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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLL, MASS.

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BIOLOGICAL BULLETIN

ON THE NORTH AMERICAN MARINE TRICLADS.

DR. J. WILHELMI.

During the last fifty years ten marine species of triclads were found on the east coast of North America:

Procerodes wheatlandi Girard (according to Verrill = *Procerodes ulvæ* (Oe.), according to Curtis = *Gunda segmentata* Lang).
Procerodes frequens Leidy (according to Verrill = *Procerodes wheatlandi* Gir.).
Fovia (*Vortex*) *warreni* (Gir.).
Fovia (*Planaria*) *grisea* (Verrill).
Fovia affinis (Oe.) (var. *warreni* and var. *grisea*).
Fovia (*Planaria*) *littoralis* (Verrill).
Bdelloura (*Vortex*) *candida* (Gir.) (= *Bdelloura parasitica* Leidy = *Planaria limuli* v. Graff).
Bdelloura propinqua Wheeler.
Bdelloura rustica Leidy.
Syncoelidium pellucidum Wheeler.

The identifications for the most part of these are quite doubtful, because of the incomplete description; also the identifications of these species with European species, given by some authors, are untenable. I studied these North American species in the most important localities and in the following paper I shall give a short notice of my results. I am not concerned with the Bdellouridæ (genera *Bdelloura* and *Syncoelidium*), living on *Limulus*, because these have already been well described by Wheeler.[1] *Bdelloura rustica*, a free-living form, described by Leidy[2] cannot be identi-

[1] Wheeler, W. M., "*Syncoelidium pellucidum*, a New Marine Triclad," *Journ. Morph.*, Boston, 1894, pp. 167–194, Pl. 8.

[2] Leidy, T., "Helminthological Contributions," No. 3, *Proc. Acad. Nat. Sc.*, Vol. V., Philadelphia, 1850/51, pp. 242, 243, 289.

fied as such and doubtless it does not belong to the genus *Bdelloura*. Also I have published a paper[1] on the larvæ of planarians, named by Agassiz[2] *Planaria angulata* and the probable confusion of these with young Bdellouridæ. The following communication concerns only the free-living marine triclads.

Girard[2] described in 1850 a marine triclad, found near Manchester (Massachusetts Bay) under the name *Procerodes wheatlandi* n. gen. n. sp. The same animal was found by *Leidy* (9) in 1855 near Point Judith, R. I., and was described as *Procerodes frequens* n. sp. Neither species was investigated in relation to the genital apparatus and nevertheless Verrill (10), in 1873, identified them with the North European species *Gunda ulvæ* (Oe.).[3] Girard[8] called the first species by its old name *Procerodes wheatlandi* but the second and the third (European) species he classed with the Rhabdocoelidæ (!) under the name of *Neoplana* n. g. *frequens* and *N. ulvæ*. Curtis[1] supposed that Verrill's *Procerodes ulvæ* (*Procerodes wheatlandi* + *Procerodes frequens*) may be identical with the South European *Procerodes* (*Gunda*) *segmentata* (Lang).

Without mentioning the mistake of Girard, there are two questions to answer: (1) Is *Procerodes wheatlandi* (*Procerodes frequens*) identical with one of the European triclads *Procerodes ulvæ* or *Procerodes segmentata?* (2) Must the European genus *Gunda* O. Schm. (+ *Haga* O. Schm.) be classed with the American genus *Procerodes?*

In relation to the last question Bergendal[4] correctly mentioned that for the present the well-described genus *Gunda* must be preferred to the insufficiently described genus *Procerodes*. Böhmig[5]

[1] Wilhelmi, J., "Über *Planaria angulata* Müller," *Zool. Jahrb.*, Abth. Sytematik, 26. Bd., 1907, 10 pp., 1 Taf.

[2] Agassiz, A., "On the Young Stages of a Few Annelids," *Ann. Sc. N. H.*, New York, 8. Bd., 1866, pp. 306–309, Taf. 1, figs. 1 u. 2.

[3] Oersted, A. S., "Forsog til en ny Classification of Planarierne (Planarica Dugès) etc.," *Kroyers Naturh. Tidsskrift*, IV., 1843, p. 551.

[4] Bergendal, D., "Studier öfver Turbellarier. 2. Om Byggnaden af *Uteriporus* Bgdl. jämte andra bidrag till Tricladernas anatomi." *Fysiogr. Tällsk Lund Handl.* (2), Bd. 7, 1896. — Ueber drei Tricladen aus Punta Arenas und umliegender Gegend," *Zool. Anz.*, 22. Bd., 1899.

[5] Böhmig, L., "Turbellarienstudien: *Tricladida maricola*," *Zeitschrift f. wiss. Zool.*, 81. Bd., 1896.

referred to the same question : " Die von Girard gegebene Charakteristik des Genus *Procerodes* ist eine sehr oberflächliche, sie bezieht sich nur auf das Extérieur, während die Beschreibung und Abbildungen O. Schmidt's (*Gunda*) genügend kennzeichnende sind. . . . Mit Rücksicht auf die grosse Uebereinstimmung, welche sich hinsichtlich der Form zwischen *Procerodes* und der überwiegenden Mehrzahl der *Gunda*-Arten ergibt, mit Rücksicht weiterhin auf den Umstand, dass wenigstens eine sichere *Gunda*-Species an der Nordamerikanischen Küste beobachtet wurde ("but it was not"), acceptiere ich die *Girard'sche* Bezeichnung, obwohl der Copulations-apparat von *Pr. wheatlandi* total unbekannt ist und den Zweifeln, die Bergendal bezüglich der Identität von *Procerodes* und *Gunda* äussert, eine Berechtigung nicht abgesprochen werden kann. Mit Sicherheit lässt sich diese Frage nur durch die Untersuchung der Originalexemplare von *Pr. wheatlandi* entscheiden, . . ."

I studied the above mentioned North American [1] and European [2] species and found that following Verrill the genus *Gunda* O. Schm. because of the agreement of the genital apparatus of *Procerodes wheatlandi* with this of European *Gunda*-Species must be classed with *Procerodes*. But *Procerodes wheatlandi* (and *frequens*) is not identical with *Procerodes* (*Gunda*) *ulvæ* although they closely resemble one another. Also Curtis' [1] supposition given in a short notice, " The occurrence of *Gunda segmentata* in America," that *Procerodes wheatlandi* may be identical with the South European species *Gunda segmentata*, and not, as Verrill supposed, with *Procerodes* (*Gunda*) *ulvæ*, is a mistake. *Procerodes segmentata* [3] is quite free from pigment and therefore, setting aside the different forms of the head will be easily distinguished from the North American *Procerodes wheatlandi* and the North European *Procerodes* (*Gunda*) *ulvæ*.

Nearly contemporary with the description *Procerodes wheat-*

[1] Collected at Cuttihunk (Elizabeth Islands) and Newport, R. I. ; through the courtesy of Professor Curtis I obtained also the original material of his *Gunda segmentata* from Sandwich, Mass., where I myself unsuccessfully sought for these species. I found it in Buzzards Bay, near Sandwich, summer 1907.

[2] I collected it at Travemünde and Copenhagen in September, 1906.

[3] I collected it during the last three years at Naples and many other localities of the Mediterranean Sea.

landi Girard (3) described a second new marine triclad from Boston Bay, *Vortex warreni* n. sp., for which later he established the new genus *Fovia* (7). Verrill (10) in 1873–74 described the same species as *Planaria grisea* n. sp. and later as *Fovia littoralis* n. sp. (11). The descriptions of these species and of the genus *Fovia* are insufficient. Already in 1857 Stimpson [1] classed the European *Planaria affinis* Oe. with the genus *Fovia* and Verrill (12) identified the preceding North American species with Stimpson's *Fovia affinis* (Oe.) as varieties *warreni* and *grisea*. Girard (3) meanwhile cites *Fovia warreni* as a marine triclad (with anus) and classed the identical *Fovia grisea* with his new rhabdocœlid (!) genus *Neoplana*. From the North European *Fovia affinis* I studied the only three existing individuals of the Museum of Bergen and showed [2] that probably it is a fresh-water form, which sometimes occurs also in brackish and sea-water, perhaps *Planaria torva* Müll. The American species *Fovia warreni* and *grisea* (littoralis [3]) spoken of by Verrill as *Fovia affinis* (Oe.) belong to only one species of the genus *Procerodes*, which must be designated *Procerodes warreni* (Gir.); the variations of color not being greater than usual in sea- and fresh-water-planarians, do not allow of forming separate varieties; the genus *Fovia* must be included in the genus *Procerodes*. Girard (4, 7, 8) described it as viviparous: the larvæ resemble the adult animal, but its anterior end is less truncated. Eyes are still absent in the larvæ, but the position of these is indicated by two transparent spots; a canal in the middle of the body is interpreted as the alimentary tube.

These "larvæ" are protozoans (*Hoplitophrya*), living in the cavity of the pharynx and in the intestine of triclads. M. Schultze [4] found them in *Procerodes* (*Planaria*) *ulvæ* of the Baltic Sea and described them under the name of *Opalina uncinata*. I myself found them in large numbers in *Procerodes segmentata*

[1] Stimpson, W., "Prodromus, etc., *Proc. Acad. N. Sc. Philadelphia*, 1857.

[2] Wilhelmi, J. "Über *Planaria affinis* Müller." *Bergens Museums Aarbog*, 1907, Nr. 4.

[3] I collected them at Woods Hole and neighborhood and at Massachusetts Bay, summer, 1907.

[4] Schultze, M., "Beiträge zur Naturgeschichte der Turbellarien," 1. Abtheilung, Greifswald, 1851.

(from the Bay of Naples, the Mediterranean and Black Sea), in *Procerodes ulvæ* (from the Baltic Sea) and also in the free-living North American marine triclads. They live principally in the cavity and the ramifications of the gut without damaging the host. The transparent spots called by Girard the first traces of the eyes, correspond to the organs of attachment and the alimentary tube answers to the nucleus of the *Hoplitophrya uncinata*.

With a detailed study of these Prof. M. M. Metcalf is occupied; at present he has given some notices on its excretory organs.[1]

I shall give in my monograph of the marine triclads an anatomical and histological description of these free-living North American marine triclads.

I append the list of the places on the east coast of North America, where these free-living triclads have been found, and indication of the corresponding literature.

Bay of Fundy, *Procerodes wheatlandi* (*ulvæ*) 1893 Verrill (12).

Eastport, Me., *P.* (*Fovia*) *warreni* 1893 Girard (8).

Grand Manan, N. Br., *P. wheatlandi* 1854 Girard (6).

Grand Manan, N. Br., *P.* (*Fovia*) *warreni* 1893 Verrill (12).

Casco Bay, Me., *P. wheatlandi* (*frequens, ulvæ*) 1873 and 1893 Verrill (10) (12).

Casco Bay, Me., *P.* (*Fovia*) *warreni* 1873 Verrill 1893 (10).

Casco Bay, Me., *P.* (*Fovia*) *warreni* (*grisea*) 1893 Verrill (12).

Cape Elizabeth, Me., *P.* (*Fovia*) *warreni* (*affinis*) 1873 Verrill (10).

Gloucester, Mass., *P. wheatlandi* (*ulvæ*) 1893 Verrill (12).

Manchester, Mass., *P. wheatlandi* 1850 and 1851 Girard (2) (5).

Beverly, Mass., *P.* (*Fovia*) *warreni* 1893 Verrill (12).

Chelsea Beach, Mass., *P.* (*Fovia*) *warreni* 1852 Girard (7).

Boston Harbor, Mass., *P.* (*Fovia*) *warreni* 1850 Girard (4).

Sandwich, Mass. (Cape Cod Bay), *P. wheatlandi* (*Gunda*) *segmontata* 1900–01 Curtis (1).

Woods Hole, Mass., *P.* (*Fovia*) *warreni* 1873–74 Verrill (10).

Woods Hole, Mass., *P. wheatlandi* (*ulvæ*) 1893 Verrill (12).

Vineyard Sound, *P. wheatlandi* (*frequens*) 1855 Verrill (10).

[1] Metcalf, M. M., "The Excretory Organs of *Opalina*," Part II., *Arch. f. Protistenkunde*, 10. Bd., 1907.

Newport, R. I., *P. wheatlandi* (*ulvæ*) 1893 Verrill (12).
Point Judith, R. I., *P. wheatlandi* (*frequens*) 1855 Leidy (9).
Watch Hill, R. I., *P.* (*Fovia*) *warreni* (*grisea*) 1872 Verrill (10).
New Haven, Conn., *P. wheatlandi* (*frequens*) 1873 Verrill (10).
New Haven, Conn., *P. wheatlandi* (*frequens, ulvæ*) 1893 Verrill (12).

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1. '00 The Occurrence of Gunda segmentata in America. Biol. Bull., Boston, Vol. 2, 1900–01, p. 331.

Girard, Ch.

2. '48–'51 Several New Species of Marine Planariæ of the Coast of Massachusetts. Proc. Boston Soc. N. Sc., Vol. 3, 1848–51 (1850), p. 521.
3. '50 A Brief Account of Fresh-water Planariæ of the United States. Ibid., 1850, p. 264.
4. '50 Observations upon Planarian Worms. Ibid., 1850, pp. 363–364.
5. '51 Di Planarien und Nemertinen Nordamerikas. Kellers und Tiedemanns Nordamerikanische Monatsberichte für Natur- und Heilkunde. Philadelphia 1851, 2 Bd., p. 4.
6. '54 in: W. Stimpson, Synopsis of the Marine Invertebrata of Grand Manan, or the Region about the Mouth of the Bay of Fundy, New Brunswick. Smithsonian Contrib. to Knowledge, 6 Bd., Washington, 1854, pp. 27, 28.
7. '54 Description of Two New Genera and Two New Species of Planaria. Proc. Boston Soc. N. H., Vol. 4, 1857–54 (1852), p. 211.
8. '93 Recherches sur les Planariés et Némertiens de l'Amérique du Nord. Ann. Sc. Nat., Vol., 15, 1893.

Leidy T.

9. '55 Contribution Towards a Knowledge of the Marine Invertebrate Fauna of the Coast of Rhode Island and New Jersey. Journ. Acad. N. Sc. New Ser., Vol. 3, 1855, p. 143.

Verrill, A. E.

10. '73 Report upon Invertebrate Animals of Vineyard Sound and the Adjacent Waters, with an Account of the Physiological Characters. United States Commission of Fish and Fisheries, Commission's Report for 1871 and 1872, Washington, 1873, pp. 325, 332, 477, 480, 488, 633.
11. '74 Results of Recent Dredging Expeditions on the East Coast of New England. Amer. Journ. Sc. and Arts, New Haven, 1874, Vol. 7, p. 132.
12. '93 Marine Planarians of New England. Trans. Conn. Acad., Vol. 8, 1893, pp. 118–127.

Marine Biological Laboratory,
Woods Hole, Mass., August, 1907.

A CONVENIENT PARAFFINE BATH FOR INDIVIDUAL USE.

J. F. McCLENDON.

Paraffine baths may be classified according to source of heat, into oil, gas and electric; and according to mode of regulation of temperature into fixed, regulated by attendant and self-regulating. In the self-regulating baths, the amount of heat generated may be variable or the loss of heat variable. In using oil and gas there is more danger from fire than in using electricity. Baths with a fixed temperature regulation can be used only in rooms of approximately constant temperature, and their readjustment by an attendant is inconvenient. Apparatus for controlling the amount of heat generated is too delicate and expensive to be put in the hands of all students. There only remains the electric bath, self-regulated by varying radiation of heat. Several forms of baths using this method have been used, and the form herein described differs from them chiefly in size, shape and material.

The bath consists of a rectangular concrete trough having inside measurements of 21.5 cm. in length, 11 cm. in width and 9.5 cm. in depth, and a general thickness of about 3.5 to 4 cm. In making these troughs I molded the inside with some old pieces of glass and left the glass in as a lining (in case this was not done the inside might be painted or left bare — the paraffine would not soak through so long as the outside is below its melting point). The trough is filled with paraffine of melting point one degree or more (3 in present instance) higher than that used in imbedding. The top is closed by a thin piece of wood in the center of which is a flush lamp socket with opening downward. Into this socket is screwed a four candle power "incandescent" lamp of the spherical type. The lamp reaches about to the bottom of the trough, while the socket projects upward above the top of the bath and is connected by small flexible wires or lamp cord to a 110-volt circuit. In the wooden top near the lamp are cut holes of suit-

able size and shape for sinking receptacles for imbedding dishes, paraffine pitchers, etc. For holding small vials I sunk larger ones through the wooden top and filled the space between the two with lubricating oil to prevent cooling by currents of air when the bath was opened. A flat tile would be useful to cover each side of the bath, avoiding the lamp socket.

The temperature regulation is more perfect than in any other small bath I have seen. The heat of the lamp melts the adjacent paraffine until the radiation equals the heat supplied, and convection currents keep the melted portion at constant temperature. When the temperature of the room rises, more of the paraffine in the trough melts and increases the radiating surface. When the temperature of the room falls, part of this paraffine congeals and decreases the radiating surface. The paraffine in the imbedding dishes is always kept melted unless they have been set too deep or too far from the light. The bath is intended to be fireproof, but if space is the determining factor it should be made of metal, in which case a higher candle power lamp should be used. The trough might be made of glazed earthenware with double side walls, the spaces between these walls opening downward and the outer walls projecting below the bottom of the bath, thus insuring an air space beneath.

Zoölogical Laboratory,
University of Missouri.

THE SPAWNING HABITS OF CHROSOMUS ERYTHROGASTER RAFINESQUE.*

BERTRAM G. SMITH.

While a student at the University of Michigan during the years 1904–1907 I acted as assistant in zoölogy and when accompanying Professor Jacob Reighard on field excursions had many opportunities to observe, under his guidance, the breeding habits of minnows and other fishes. This familiarity with the unpublished work of Professor Reighard made it possible for me to make the observations recorded below, which are published with his permission.

About the middle of May, 1907, I found the minnow *Chrosomus erythrogaster* Rafinesque very abundant in a small brook near Lake Forest, Ill. As the males were in breeding dress I seized the opportunity to make some observations on the spawning habits.

Habitat. — The brook flows through a large open pasture and also through woodland; the minnows were far more numerous in that portion of the brook which is in the open field. Here the stream meanders through a shallow valley, over a pebbly or sandy bottom. In places it is so narrow that one can readily step across it; elsewhere it expands into pools or shallow rapids not more than six feet wide. In the rapids the water is seldom more than two or three inches deep; in the pools it may reach a depth of one or two feet. The current is quite rapid; even in the pools there is little quiet water.

Chrosomus is by far the most abundant fish in this brook. Schools of from a dozen to several hundreds abound, while of other fishes only a few large suckers, and an occasional *Rhinichthys*, *Semotilus* and *Cottus*, were observed.

Sexual Dimorphism. — The bright colors of the males were scarcely noticeable when the fishes were in the water and viewed obliquely from above; but when removed from the water the

* Contributions from the Zoölogical Laboratory of Syracuse University.

males were found to be marked on each side of the abdomen with a broad longitudinal stripe of the most vivid and brilliant scarlet that I have ever seen. This stripe starts just back of the operculum and runs immediately below the lower of the two lateral dark bands (see Fig. 1) and parallel to it, reaching almost to the caudal fin. There is also a small red spot just below the

FIG. 1.

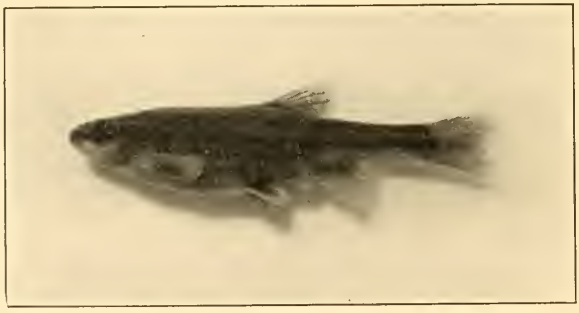

FIG. 2.

FIG. 1, male, and Fig. 2, female specimens of *Chrosomus erythrogaster* Rafinesque, life size.

mandible on each side. In some specimens the lateral bands of red are faint except just behind the operculum. In the more highly colored males the entire abdomen is covered with red, and there is a red spot in the anterior part of the root of the dorsal fin. The pectoral and pelvic fins, and the anal fin, are bright lemon yellow; the dorsal fin and tail are faintly marked with pale yellow. There is a small spot of yellow on the ventral side of the body at the base of the caudal fin, and another in the gular region.

The males possess "pearl organs" (small conical thickenings of the epidermis), occurring over practically the entire surface of the body, head, tail and fins. They are especially numerous and well developed on the dorsal and lateral surfaces of the body, on the opercula, over the entire surface of the tail, and on the dorsal surfaces of the pectoral fins; they are large, but comparatively few in number, on the dorsal surface of the head. They are much smaller on the ventral surface of the abdomen, and entirely lacking about the mouth and at the tips of the fins.

As seen under the microscope, a pearl organ from the body of a male is a sharply pointed transparent spine, rising from a broad base and pointing obliquely backward, occurring as a thickening of the epidermis over the middle of the posterior margin of each scale. Usually there is only a single pearl organ for each scale, but sometimes two spines occur close together. Smaller rudimentary pearl organs, each consisting of a papilla or thickening of the epidermis without a spine, also occur, one or more over each scale and usually near its center.

The pectoral fins of the male are much larger and stronger, in proportion to size of body, than those of the female. As shown by average measurements, the pelvic fins of the male are also proportionally larger than those of the female, but the difference is not so great as in the case of the pectoral fins.

The females are on the average slightly smaller than the males (see Figs. 1 and 2), but have the abdomen distinctly swollen. There is a small patch of red just back of the operculum. They have the same yellow coloration as the males, but the color is comparatively faint. They possess only rudimentary pearl organs, hardly visible except under the microscope.

A notable exception to the usual sexual dimorphism was found in the case of one unusually large female with well developed ovaries and eggs, but with the secondary sexual characteristics of the male all very well marked.

I had no opportunity to study the color changes and the history of the pearl organs throughout the year.

Sex Ratio. — In order to determine the numerical proportion of the sexes, representative samples of several schools were taken on several different days. The tabulated results are as follows:

Date of Capture.	Mode of Capture.	Number in Catch.	Females.	Males	Ratio.
1. May 17.....	Hoop net	6	2	4	1 : 2
2. " 29.....	Single sweep of seine	119	9	110	1 : 12.2
3. " 31.....	Hoop net	80	14	66	1 : 4.7
4. June 14.....	Hoop net	15	4	11	1 : 2.7
	Total..	220	29	191	1 : 6.5

In lots 1, 3 and 4 the sexes were determined by dissection. Since in only one of these specimens the secondary sexual characters were those of the opposite sex, the probable error in determining the sex by the external characters is a negligible quantity. In lot 2 the sexes were determined by the external characters.

These results are sufficient to indicate a decided preponderance in the number of males on the spawning ground at the spawning season, though not necessarily at other times or places.

Spawning Behavior. — The casual observer will find schools of *Chrosomus* occurring in the pools and the deeper portions of the swift water of the brook. On quietly approaching the stream, one will often notice a splashing of the water of the shallow rapids, scarcely distinguishable from the ordinary rippling of the current. Further observation will reveal the fact that in places the water is alive with tiny fishes, struggling and crowding one another in water so shallow that the suface is violently agitated; occasionally a mass of wriggling fishes will flash into view at the very surface, or crowd splashing upon the pebbly shore, where some may be left stranded, later to struggle back into the water.

When alarmed, the fishes almost invariably swim up stream from the shallows. In the deeper water they gradually recover from their fright; those that have hidden amongst the water vegetation or under the shadow of overhanging banks, rejoin the school. Together, moving in unison like a flock of sheep, they surge slowly down stream; but before they reach the shallow water again, they face about with an eddying movement, and swim up stream. This circuit is made repeatedly, the school drifting each time a little farther down stream toward their proper spawning grounds. The behavior of the fishes from the time they are frightened away from the shallows, until they resume

active spawning operations on the same ground, forms a series of events which occur on different occasions with unfailing regularity.

On the up stream journey, occasional isolated cases of spawning occur. Several males pursue one female ; as the foremost males gain a position alongside the female, the flight and pursuit attain almost lightning-like rapidity. At length two males spawn with the single female as follows: One on each side presses the side of his head against that of the female, all three facing up stream. The two males then crowd laterally against the female, held between them (see Fig. 3); their entire flanks are thus pressed against the sides of the female. While the males are in this position, a rapid vibration of their bodies occurs. The wave of pressure begins at the anterior end of the body and passes backward as a sidewise undulating movement. Other males may attempt to crowd in. So far as observed, the female remains passive. The entire performance occurs so quickly that the details are made out only after considerable practice in observation.

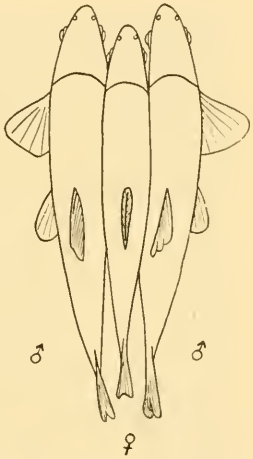

FIG. 3. *Chrosomus* in spawning position. Natural size.

Spawning under these conditions seems to be attended with difficulty. The act of clasping lasts but for an instant; the males seem unable to keep their position after the impetus of their rush up stream is overcome by the current, for swimming movements are necessarily discontinued during spawning. It is doubtful if spawning in the open, without contact with the bottom, is very effective ; milt was not extruded in sufficient quantities to be seen. Although the eggs (almost transparent, with light-brown yolk, always difficult to see in the water) could not be seen, it was evident that some were extruded, for immediately after the spawning other fishes poked their noses into crevices between pebbles, just down stream from where the spawning occurred, evidently trying to get at the scattered eggs to eat them.

As the school in its circling movements nears the shallows, a

few of the foremost fishes, with shy, frightened movements, dart rapidly down stream through the swift water of the regular spawning grounds; after the first reconnoissance they hurry back to rejoin the main body, and the entire school moves up stream. This is repeated several times; each time the band of more venturesome fishes is increased in number, and a little later, on the up-stream journey, they stop for a time to spawn on the pebbly margin of the stream. Soon the entire school, in a compact body, is spawning on the shallows. With their swift movements

FIG. 4. Spawning grounds of *Chrosomus*. The shallow water near shore is occupied by a school of several hundred *Chrosomus;* some of these may be indistinctly seen, in spawning position, at the surface of the water in the center of the picture.

and brilliant colors, this writhing mass of several hundred fishes affords a truly remarkable spectacle.

Persistent attempts to photograph the spawning operations met with poor success, a result due in part to the lack of a suitable camera. Figs. 4 and 5 are reproductions of two of the many views taken.

This procedure of spawning *en masse* in shallow swift water

may be regarded as the normal spawning behavior of these fishes. All the fishes face up stream, but their swimming movements are only sufficient to keep them from being swept down stream by the current. In the confused and wriggling mass it is difficult to distinguish the movements of individual fishes; but it may be seen that many males crowd alongside a single female, and those immediately in contact with her may occasionally be seen in the spawning position described as occurring in the deep water.

Gradually the school crowds closer against the shore, rasping

FIG. 5. Spawning grounds of *Chrosomus*. Spawning fishes crowding up on the island of gravel, may be seen near the center of the picture. In the foreground the water is alive with fishes.

over the pebbles and wriggling into water so shallow that their dorsal surfaces are exposed. In these situations small groups become segregated from the main mass; their progress is impeded by the pebbles, so that accurate observation becomes an easy matter. Typically, two males lie alongside a single female; the group becomes wedged in between large pebbles and in close contact with the bottom, so that the males are enabled to keep

their position indefinitely, and lie for many minutes motionless except for the frequently occurring rapid vibration of their bodies. The spawning position is exactly the same as that observed in the open, but the performance is greatly prolonged, and spawning doubtless much more effective. Frequently, groups of four or even more fishes were seen lying alongside, with their bodies in close contact; occasionally a group of six, composed of two trios, each consisting of a single female, spawning with two males.

One marked variation from the usual spawning method was observed. A female in very shallow water spawned with a single male, which crowded her laterally against some pebbles and curved his tail up over her body, thus holding her firmly against the bottom. His body vibrated rapidly and the water became cloudy with milt.

Some further observations on the spawning behavior were obtained by the study of a small school which occupied an abandoned dace's nest. When first observed, this school consisted of a dozen or more males, crowded closely together in the hollow of the nest. Occasional females approached shyly from down stream, singly, or two or three in succession; sometimes darting rapidly, as if excited or frightened, up the middle of the stream; again keeping close to the shore, and occasionally seeking cover. The arrival of a female created intense excitement amongst the males in the nest. They immediately crowded around her, pressing alongside and against her in the hollow of the nest. After spawning, the males devoted considerable attention to prying about between the pebbles as if searching for eggs. Sometimes the males, in a body, left the nest and returned to it repeatedly, moving in an excited manner as if seeking to entice or drive a female into it. The nest was evidently an especially favorable place for spawning.

Eggs in various stages of development were obtained by scooping up the gravel of any of the spawning grounds.

Breeding Season. — The earliest observations were made on May 17. At this time the bright colors of the males were well developed and spawning was in progress, but how long it had been going on is not known. The latest observations were made on June 14; then the schools of fishes spawning on the shallows

were small, while in the pools below were larger schools which took no interest in spawning, and were comparatively inactive. The males of these latter schools had nearly lost their bright colors. Evidently the breeding season was nearly over. At this time many of the eggs had hatched out.

During this prolonged breeding season, extending over fully a month, the stream was frequently swollen by freshets, which presumably interfered with the spawning operations.

Discussion. — As shown by the unison of their circling movements when not engaged in spawning, and by the fact that they occur in schools even after the spawning season is over, these fishes possess a strong gregarious instinct. The two conspicuous longitudinal dark stripes on the sides of the bodies of both sexes probably serve as recognition marks.

The distribution of pearl organs over practically the entire body of the male affords a roughened surface which during spawning aids him in keeping his position beside the female. The function of the pearl organs of the Cyprinidæ was first discovered and described by Reighard ('03). The structure of pearl organs has been figured by various writers.

The greater size and strength of the pectoral fins of the male as compared with the female, and the fact that the dorsal surfaces of these fins are unusually well provided with pearl organs, indicate that these fins are of use to the male in holding the female. The male, coming up from the rear, doubtless interlocks his pectoral fin between the pectoral fin and the body of the female, though it was impossible to see this.

The excessive number of males present on the spawning grounds is perhaps correlated with the method of spawning, since two males spawn with a single female.

The observable factors in sex recognition are: the brilliant colors and aggressive bearing of the male; the dull colors, swollen abdomen, and timid, hesitating movements of the female.

It is significant, in its bearing on current theories of secondary sexual characteristics, that the female showing the secondary sexual characters of the male was an unusually large and apparently vigorous specimen; but it would be unwise to base conclusions on a single instance.

The total absence of combat amongst the males accords with the necessity for coöperation in the spawning act. Competition amongst the males is limited to the struggle to get a position next to the female; in this contest the swiftest and strongest succeed.

In general the method of spawning is adapted to fishes with gregarious habits, and would seem hardly likely to be developed amongst fishes whose usual habit of life is solitary.

The pebbles amongst which the eggs lodge afford them some protection, though it appears that some of the eggs are eaten by the parent fishes. The gradual subsidence of the water during the latter part of the season must have left some of the eggs to perish on the shore. That the method of spawning is, in general, a successful one is attested by the abundance of these fishes in this brook.

A comparison with the habits of other inland fishes would be impossible without reference to the unpublished work of Professor Jacob Reighard.

Syracuse University,
April, 1908.

LITERATURE.

Reighard, Jacob.

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THE PROCESS OF EGG-MAKING IN A TREMATODE.

EDWIN LINTON.

An outline of the process here described was published by me in connection with the description of the species *Epibdella bumpusii*, an ecto-parasite of the sting ray (*Dasyatis centrura*).[1]

Since opportunities of witnessing this instructive phenomenon are rare, and descriptions are wanting, even in the larger text-books, and, further, since the knowledge of the process has proved to be of great value in explaining to students the anatomy of the reproductive apparatus of the trematodes, I venture to publish a description of the process as I have seen it.

Every teacher of zoölogy is familiar with the anatomy of some typical trematode. The description of the relations of the various parts of the complicated reproductive machinery is about as far as one is apt to go in lecturing to a class, unless he has happened to see the machinery in operation, or, possibly, has had access to a description of the physiological working of that machinery. If what I have here written will help to vivify now and then a lecture on the reproductive apparatus of the trematodes I shall be repaid for the small trouble it has been to write out this narrative.

The species *E. bumpusii* is peculiarly well adapted for the study of the process of egg-making. The animal is small enough to allow of satisfactory study with low magnification, and is, withal, transparent enough to permit rather minute details to be clearly seen. Furthermore, the animal can be kept for several hours in sea water under slight pressure without impeding the normal action of the reproductive organs.

Anatomy.

In this description only the anatomy of the reproductive organs is considered. Reference to the figures and the explanation of the same will be necessary in order to follow this description.

As in most trematodes the individuals in this species are her-

[1] Bulletin U. S. Fish Commission for 1899, pp. 286-7, figs. 11-15.

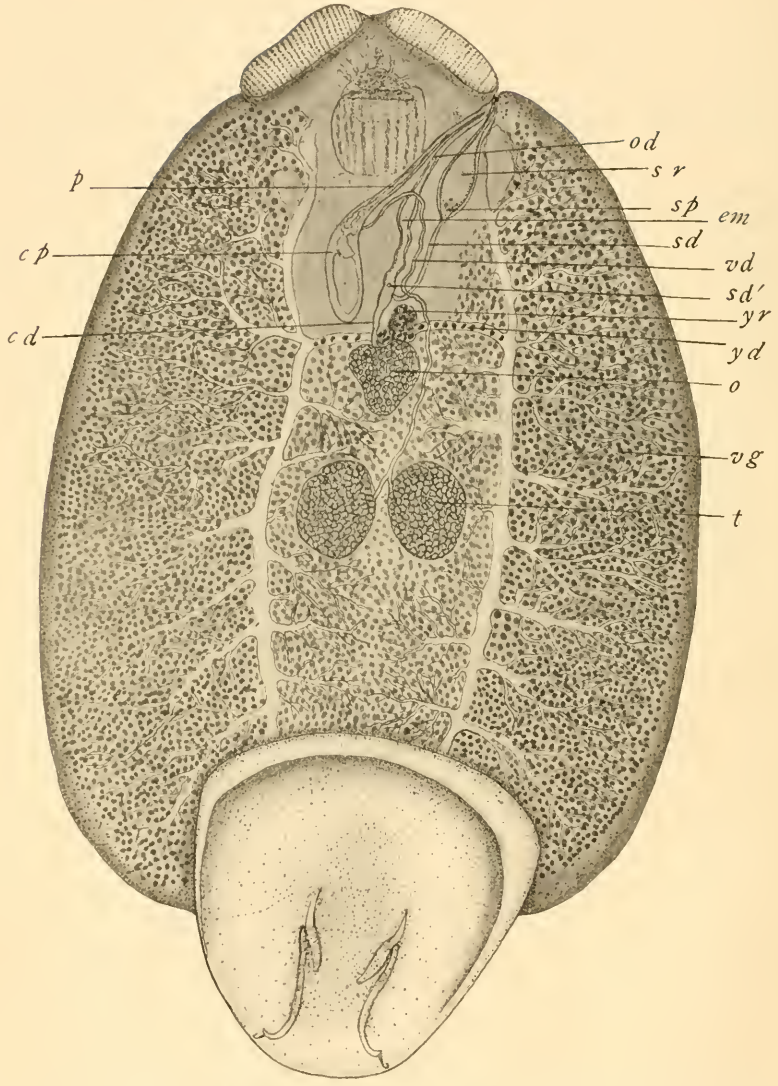


FIG. 1. *Epibdella bumpusii* Linton, ventral view; vitellaria partly diagrammatic. Actual diameter at level of testes 4.5 mm.

maphroditic. The male genitalia consist of an eversible cirrus (*p*), a cirrus-pouch (*cp*), in which is the seminal vesicle (*sv*). A vas-deferens (*vd*), which follows a somewhat tortuous course, may be easily traced from the seminal vesicle to the two testes

(*t*), which are subglobular in shape and lie near each other transversely placed and a little in front of the middle of the body. The cirrus pouch pulsated rhythmically and continued to pulsate long after all movement had ceased in the other organs.

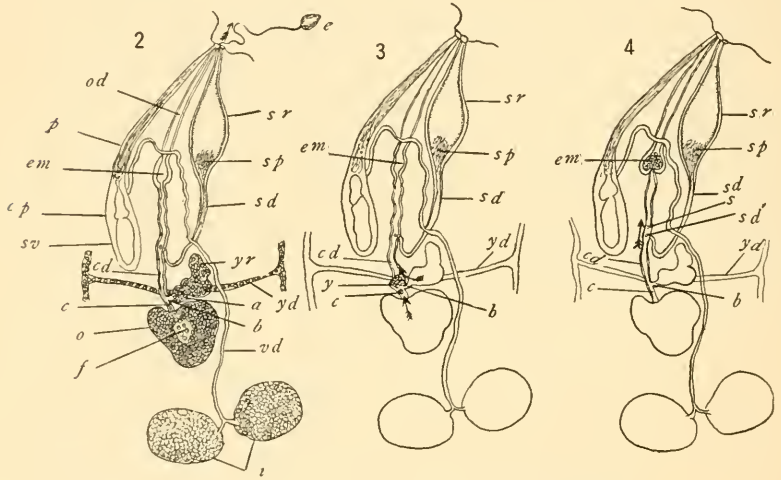
The female genitalia are more complicated. Filling the body laterally and posteriorly are the vitellaria (*vg*). These are dendritic glands whose ducts ultimately are collected into a transverse duct (*yd*) which enlarges into a vitelline reservoir (*yr*), lying a little to the left and a little in advance of the germarium (*o*). Immediately in front of the testes is the germarium (*o*), a somewhat trilobed organ lying on the median line. A short duct (*c*) passes at first dorsad then cephalad from the germarium where it joins another short duct (*ó*) coming from the vitelline reservoir (*yr*). The common duct (*cd*) then passes near the median line, cephalad to the shell mold (oötype) (*em*), which lies near the cirrus-pouch and to its left. About midway between the germarium and the oötype the common duct is joined by a minute duct (*sd*, *sd'*), which enters on its dorsal side and comes from the seminal receptacle (*sr*). The seminal receptacle lies to the left and a little in advance of the oötype.

The cells which constitute the shell-forming gland surround the common duct for some distance back of the oötype. The oviduct passes forward from the oötype, lies beside the cirrus, and opens with it in the genital notch which lies on the anterior border on the left side of the head. The vagina opens in the genital notch close to the left side of the oviduct. It leads into a capacious seminal receptacle (*sr*), from the caudal end of which the seminal duct (*sd*), above mentioned, proceeds mediad to the common duct from the vitelline reservoir and germarium.

Physiological Action of the Genitalia which are Concerned in the Process of Egg-Making.

Following are the events named in order as they were seen to occur in a specimen which was lying in sea water under a coverglass. First, a lobe (*a*) of the vitelline reservoir (*yr*) contracted vigorously thereby emptying itself of a definite mass of the coarsely granular vitelline substance with which it was filled. As this takes place in a definite portion of the reservoir, it fol-

lows that approximately the same amount of yolk is thus discharged at each contraction. The mass of yolk is propelled with comparative rapidity along the short vitelline duct (*b*) towards the median line. This duct passes near the anterior border of the germarium (*o*), where it is joined by another short duct (*c*) from the germarium. As the mass of yolk was passing the germarium it was noticed that a number of free nucleated cells, which appeared to be lying loosely in a median area (*f*) of the germarium, were set in oscillatory motion. It was quite evident



FIGS. 2, 3, 4. Diagrams showing different stages of egg-making.

FIG. 2. An egg (*e*) has just been discharged from the oviduct (*od*). A mass of yolk for the next egg is collecting in a muscular lobe (*a*) of the yolk reservoir and is about to be ejected through the short duct (*b*) into the common duct (*cd*).

FIG. 3. The mass of yolk (*y*) has entered the common duct (*cd*) and its suction is probably an inciting cause of a germ's entering the common duct from the germ-duct (*a*). The direction of movement of yolk and germ is shown by the arrows.

FIG. 4. The mass of yolk has passed by way of the common duct to the oötype (*em*) where it has been molded into a tetrahedral shape and a shell has been deposited around it. A cluster of spermatozoa (*s*) has been injected into the common duct (*cd*) from the muscular seminal duct (*sd*) at its opening (*sd'*) and has proceeded a short way towards the oötype. The arrow shows the direction of movement of the spermatozoa. In the process of egg-making the egg is discharged from the oötype a very short time (one or two seconds) after the spermatozoa have arrived at the oötype.

that the agitation of the germ-cells was due to the passage of the mass of yolk from the vitelline reservoir. While it was

certain, from what could be made out from the mechanism of the living worm, that one result of the rush of yolk in passing the germarium (see arrow in Fig. 3 lying partly on the yolk reservoir), was to draw a germ-cell from the duct (*c*) (see arrow on germarium, Fig. 3), no cell was seen to leave its place in the germarium to enter the common duct (*cd*). The reason for this failure to see a germ-cell join the mass of yolk appeared when sections were studied. It was then seen (Fig. 5) that the germ-duct (*c*) leaves the germarium on its dorsal side, and is therefore seen only in end view when the worm is flattened out on the slide. In so much of the duct as was visible there were many free germ-cells, all of them oscillating more or less and thrown into vigorous agitation at the time when a mass of yolk was

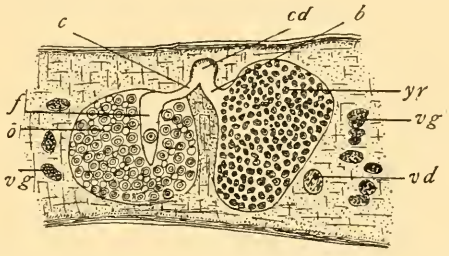


FIG. 5. Transverse section showing junction of ducts from yolk reservoir and germarium in common duct. Actual thickness of body through germarium 0.5 mm.

passing the germarium. If a cell were to leave the germarium its motion would be in a direction perpendicular to the eye, and therefore could not be detected. The portion of the duct which proceeds cephalad is somewhat concealed by the germarium. Hence here is one point in the process of egg-making, which, although perfectly obvious from the construction of the mechanism, was not actually seen. As the duct is at first perpendicular to the surface of the worm, it is obvious that the progressive motion of the germ-cell cannot be detected whether viewed from the dorsal or the ventral side.

The mass of coarsely granular yolk (Fig. 3, *y*) passes without pause and with comparative rapidity along the common duct (*cd*) to the oötype (*em*), which is simply a specialized part of the common duct. As soon as the mass of yolk reaches the oötype the passage closes by the approximation of the walls of the duct

thus forming a solid base on which the yolk rests. Against this base the mass of yolk is hammered by the walls of the oötype. Under this hammering the mass assumes a tetrahedral shape, and during the process the shell is built around it. The material for this shell is secreted by the gland which lies on each side of the common duct between the germarium and the oötype. When the shell is nearly finished, as shown by the cessation of the hammering process of the walls of the oötype, a very small and finely granular mass (Fig. 4, *s*) makes its appearance suddenly in the common duct at a point (*sd'*) approximately half way between the germarium and the oötype. This cluster of granules, which was in no case seen until it made its appearance in the common duct, travels rapidly along the common duct to the oötype. As soon as it reaches the oötype which now contains the newly encapsuled yolk mass with its associated germ-cell, there is a pause in the movements of the walls of the oötype and common duct for an instant. This pause is followed by powerful contractions of the walls of the oötype whereby the egg is forcibly ejected through the uterus (*od*) into the water.

The minute granular cluster which was seen entering the common duct immediately before the discharge of an egg, was interpreted at the time of observation to be spermatozoa, although the duct leading from the seminal receptacle to the common duct could not be seen in the living worm. It can be seen, however, in a stained specimen mounted in balsam and was satisfactorily demonstrated by means of serial sections.

Both the yolk mass and the spermatozoa appear to be propelled along the common duct by ciliary action. The egg is ejected by powerful muscular action of the walls of the oötype.

An examination of serial sections showed an interesting feature in the structure of the duct which leads from the germarium to the common duct. It is spacious at its beginning in the gland (*f*), where it appears in life as a clear space in the center of the germarium in which ripe germs could be seen oscillating whenever a charge of yolk was passing towards the oötype. The duct grows narrower distally. Indeed the duct (*c*) is shaped like a funnel. Near the point of union with the yolk duct (*b*) it is but little wider than the diameter of a single germ cell. A study of

sections makes it quite clear that the germ duct (*c*) and the yolk duct (*b*) connect in such manner that when a mass of yolk rushes along the yolk duct past the opening of the germ duct and into the common duct (Fig. 3, *b*, *c*, *y*, and arrow) sufficient suction is created to draw a germ from the germ duct.

Since the amount of yolk which is necessary for a single egg is automatically emptied into the yolk duct, the whole reflex has become adjusted with such marvellous nicety, the several parts to each other, that it seems probable that just enough suction is created to draw a single waiting germ cell from the germ duct which thus joins the passing charge of yolk.

The stimulus which causes a discharge from the seminal duct (*sd*, *sd'*) into the common duct always to take place immediately upon the completion of the process of molding the egg-shell is, of course, not obvious. The whole complicated process is an intricate nerve reflex.

Egg-making was observed to proceed actively for ten minutes or more to be followed by a short interval of rest. The time occupied in making an egg was not noted until the specimen had been under observation for two or three hours, by which time the worm had doubtless lost some of its vitality. When noted, the time from the moment at which the yolk left the reservoir until the completed egg was ejected into the water was about 40 seconds.

Summary of Events.

1. A mass of yolk leaves the yolk reservoir (*yr*).

2. As the yolk mass passes the germ duct (*c*) a germ is drawn out by the suction created by the moving mass of yolk.

3. The yolk mass and germ together pass along the common duct (*cd*) to the oötype (*ot*).

4. An egg is molded into a tetrahedral shape by a kind of hammering action of the walls of the oötype, at the same time a shell is formed, its substance being secreted by the shell-forming gland.

5. A slowing up of the action of the oötype is followed by the appearance of a minute cluster of sperm in the common duct (*sd'*, *s*).

This cluster of sperms comes from the seminal duct and passes along the common duct to the oötype.

6. A momentary pause marks the arrival of the sperm at the oötype.

7. Powerful contractions of the walls of the oötype eject the egg from the uterus into the water.

Emendation of the Description of the Species.

In the original description of this species mention was made of but two pairs of hooks on the sucker. A third pair of minute hooks, posterior to the others, was subsequently found (Fig. 1).

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Explanation of Abbreviations.

Meaning of letters which are used in more than one figure.

b. Yolk duct leading from yolk reservoir to the common duct.
c. Germ duct leading from the germarium to the common duct.
cd. Common duct leading from the junction of *b* and *c* to oötype.
cp. Cirrus-pouch.
em. Oötype.
f. Beginning of germ duct in germarium.
o. Germarium.
od. Oviduct leading from oötype to exterior.
p. Cirrus.
sd. Seminal duct leading from seminal receptacle to common duct.
sd'. Point where *sd* enters the common duct.
sp. Spermatozoa in seminal receptacle.
sv. Pulsating organ at base of cirrus-pouch.
t. Testes.
vd. Vas deferens.
vg. Vitelline gland.
yd. Yolk duct leading from vitellaria to yolk reservoir.
yr. Yolk reservoir.

FORM REGULATION IN CERIANTHUS ÆSTUARII.

C. M. CHILD.

During 1905–6 it was my privilege to enjoy for several months the facilities afforded by the laboratory of the San Diego Marine Biological Association at La Jolla, California. During this time the work which forms the subject of the present paper, together with other work to appear later was accomplished. I take this opportunity of expressing my appreciation of the kindness of Professor W. E. Ritter and the other members of the association in granting me the privileges of the laboratory.

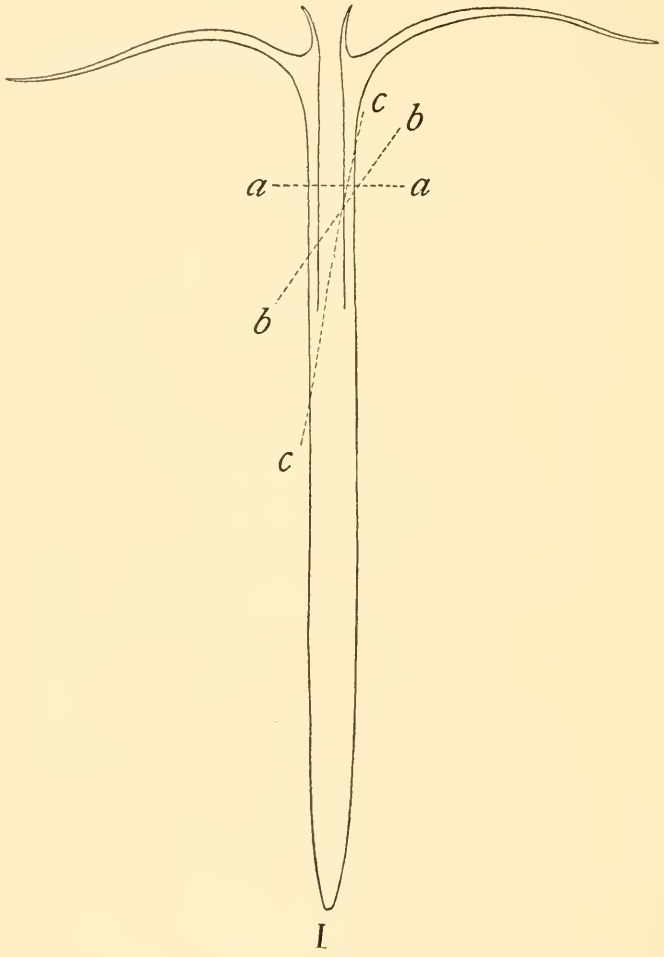
In 1902–3 I made a study of the process of form-regulation in *Cerianthus solitarius* at Naples, the results of which have already appeared (Child, '03*a*–'05*b*). In order to test and compare the results obtained with that species an extensive series of experiments was performed with *Cerianthus æstuarii* (Torrey) which occurs in abundance on the tide-flats of Mission Bay near La Jolla. For the name of the species I am indebted to Professor H. B. Torrey, as his work on the species has not yet appeared.

As regards size and general appearance *C. æstuarii* does not differ widely from *C. solitarius*. It is, however, more delicate in structure, the body-wall and especially the muscular layer being much thinner than in *C. solitarius*. In consequence of this characteristic specimens which are all well distended with water often appear more or less translucent. The length of the animal varies greatly according to the degree of distension. Fig. 1 will serve to give an idea of the usual shape.

In general the results of my experiments with this species confirm in all essentials those obtained with *C. solitarius*, so that an extended account of most of the experiments is unnecessary. But the results of experiment differ in some respects in the two species, and these differences, being due largely to the differences in the character of the tissues, possess a certain interest.

C. æstuarii, like *C. solitarius*, inhabits burrows whose walls consist of slime, nematocysts and fine sand or mud. Like *C. soli-*

tarius also it is capable of living for months in clear water in the laboratory, though, as will appear below, certain interesting modifications of shape and proportions appear under these conditions. The description of structure and habits given for *C. solitarius*



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(Child, '03*a*, p. 239 et seq.) will apply in general to this species, but it may be noted here that *C. æstuarii* is more sensitive to external stimuli than *C. solitarius*. Slight contact-stimuli with needles or brush produce extreme and rapid contraction when the animal is normally distended with water, though it is apparently much less sensitive when partially or wholly collapsed.

Specimens normally distended are also very sensitive to light: if kept in covered dishes sudden exposure even to diffuse daylight produces marked general contraction after a short latent period. As regards this stimulus also collapsed specimens or pieces are much less sensitive than those which are distended with water.

The distension of the body and the erection of the tentacles is accomplished in this species as in *C. solitarius* by the entrance of water into the enteric cavity, both through the mouth and through the body-wall. The entrance of water through the body-wall probably occurs at all times to a greater or less extent, but is most readily observed in pieces undergoing regulation in which the ends have closed but no mouth has formed (cf. Child, '04*b*, p. 267 et seq.).

I. The Course of Form-Regulation.

In pieces above a certain minimal size, isolated by transverse cuts, the course of collapse and inrolling of the body-wall in the region of section, the closure of the wound and the formation of new tissue are in general similar to those processes in *C. solitarius* (Child, '03*a*, pp. 244–257, Figs. 1–24). As regards the formation of new tissue at the aboral end there is a quantitative difference between the two species, the amount of new tissue formed in *C. æstuarii* being usually much less than in *C. solitarius* (Child, '03*a*, pp. 257–259, Figs. 25–31). Attention may be recalled to the fact that the marginal tentacles in *C. solitarius* do not arise at the cut surface itself, but a short distance aboral to it (Child, '03*a*, p. 252, Figs. 10–19), the first indications of their formation being a reduction in the thickness of the body-wall in this region and the formation of a crenated ridge around the end of the piece, followed by the outgrowth of a tentacle above each interseptal chamber. These processes follow a similar course in *C. æstuarii*.

One difference between the two species may, however, be noted in this connection: in *C. æstuarii* the inrolling of the body-wall is much less regular than in *C. solitarius*, and closure in the usual manner is retarded or prevented more often in the latter species because the formation of the thin membrane closing the cut end is impossible. In consequence of the lack of firmness and stiffness in the body-wall of *C. æstuarii* the pieces often assume very

irregular forms when they collapse at the time of operation. Certain parts of the margin at the cut end may roll inward to a much greater extent than others, so that the cut surfaces on the two sides of the body may fail to approximate at all and closure cannot occur. The results of experiment are therefore less constant and uniform than in *C. solitarius*, though in general similar.

As regards the inrolling of cut margins in pieces of various form, longitudinal and transverse strips, oblique pieces, etc., all that has been said regarding *C. solitarius* (Child, '04*a*) will hold for this species. Here, in fact, results may vary even more widely than in *C. solitarius* because the body-wall is much more flaccid in this species after collapse. It is possible to inhibit completely the process of restitution simply by cutting the pieces of certain shapes so that the inrolling of the body-wall will prevent approximation of the cut margins: under these conditions closure of the opening and formation of the missing parts does not occur.

The regional differences in the power of regulation are very similar in the two species (cf. Child, '03*b*). The rapidity of oral form-regulation decreases with increasing distance of the level of section from the oral end.

As regards the relations between size of the piece and regulation the same similarity between the two species obtains. The length of tentacles is distinctly not proportional to the size of the piece (cf. Child, '03*b*, Part II., pp. 3–6). In *C. æstuarii* the uncertainty as to the size of minimal pieces is still greater than in *C. solitarius*, because of the great irregularity in the process of inrolling in short pieces.

In neither species has the outgrowth of new tissue directly from a free cut surface ever been observed (Child, '04*a*, pp. 66–74, Figs. 25–31; '04*b*, pp. 276–279, Figs. 3 and 4). On a free cut surface of the body-wall exposed to the water scarcely a trace of growth of new tissue appears and no restitution of the missing parts. When, however, two cut surfaces or two parts of a cut surface, *e. g.*, the two margins of a fold at the cut end, come into contact fusion occurs between them, but no further growth takes place unless the region is subjected to tension produced by water in the enteron or in some other way. But when the region is subjected to tension proliferation and growth occur

and a thin membrane of new tissue is formed, whose size is dependent in part on the degree of tension existing. When pieces are cut in such shapes, *e. g.*, by longitudinal or zig-zag cuts, etc., that the cut margins or parts of them do not come into contact with each other, the outgrowth of new tissue does not in any case occur from the free cut surfaces. Such pieces may remain open indefinitely and fail completely to replace the parts removed.

In many cases after the growth of new tissue has begun between approximated parts of the cut surface the new tissue may gradually extend for a very short distance over the cut surface on each side of the point where it began to grow. In such cases it can readily be determined that the thin new tissue is itself under some slight degree of tension. Under these conditions growth proceeds to a certain point and then stops. Apparently the mechanical conditions in the region involved and the physical properties of the new tissue itself are important factors in determining the amount of growth. In my earlier experiments the fact was noted

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that such growth occurs to a much greater extent in *C. membranaceus* where the new tissue is much thicker and more resistant than in *C. solitarius* (Child, '04*a*, pp. 70–71). In *C. æstuarii*, where the tissues are even more delicate such growth of new tissue does not occur to any great extent. In Fig. 2 the end of a piece cut so that it remains widely open is shown and the extent of the growth of new tissue is indicated by the shaded areas in the angles of the inrolled margins. Such a piece never closes by extension of the new tissue over the whole opening, but remains indefinitely in the condition figured, unless the relation of the parts of the cut margin is altered by changes in the degree of contraction or by passive changes of the position ofthe piece, which bring new portions of the cut margins into contact or proximity. After such a change growth of new tissue may continue for a time where the angle between two adjoining parts of the cut margin is not too great, but in no case does the new tissue extend with

a free margin across wide spaces as in *C. membranaceus* (Child, '04*a*, Fig. 31).

If the piece becomes distended with water after closure of a cut end by new tissue the new tissue, which at first is scarcely visible between the margins of the old parts, increases in area, but in the absence of distension such growth never occurs. Fig. 3 on p. 277 of my earlier paper (Child, '04*b*) shows the growth of new tissue in a collapsed piece of *C. solitarius* and Fig. 4 the growth in a distended piece. The differences in *C. æstuarii* are similar.

There is then no escape from the conclusion that mechanical strain is a necessary factor in the growth of new tissue in these species. That it is the only factor, I should be the last to assert, but I think it is sufficiently clear that in its absence the *Cerianthus* material possesses no inherent power to restore missing parts.

II. The Control of Tentacle-Development in Regulation.

(*a*) *By Artificial Openings in the Body-Wall.*

After the closure by new tissue of the ends of pieces distension of the enteric cavity with water occurs before any opening into the enteron exists. The passage of the water through the body-wall is probably not a simple osmotic process but something more complex. When the mouth is present or after a new mouth is formed water may enter through it, and by these means a certain degree of distension is maintained. In my experiments with *C. solitarius* it was possible to prevent, at least in large measure, this distension by making openings in the body-wall and reopening them at short intervals (Child, '04*c*). Under these conditions the development of the tentacles is retarded or almost entirely inhibited.

The secretion of slime about the opening and the inrolling of the margins of the cut make it impossible to prevent all distension, but by removing the slime and reopening the pieces every few hours or even once a day the distension can be reduced far below the normal and the first appearance and the rapidity of development of the tentacles greatly retarded and their final size much reduced. If it were possible to eliminate all internal pressure the tentacles would undoubtedly fail to appear.

Experiments along this line on *C. æstuarii* confirm my earlier work. It was possible to control the development of the tentacle in this species within wide limits: in some experiments, for example, they attained a length of 1 mm. in the open pieces while in the controls they reached the length of 15 mm. in the same time.

(*b*) *Pieces from the Œsophageal Region.*

The œsophagus in *Cerianthus* extends a considerable distance aborally from the mouth (Fig. 1), and pieces in which the œsophagus extends through the whole length can readily be obtained. The process of closure of the cut ends in such pieces was described for *C. solitarius* in my earlier paper (Child, '04*d*, pp. 205–206, Figs. 7–15), and is similar in *C. æstuarii*. In almost all cases the cut ends of œsophagus and body-wall unite at both ends of the piece and the œsophagus therefore opens to the exterior at each end but does not communicate with the enteron, at all. In these pieces water can pass into the enteron only through the body-wall, but distension by this means does not continue indefinitely (Child, '04*d*, pp. 206, 211) and after a few days the pieces gradually collapse and never become distended again. Under these conditions the development of the tentacles begins but ceases as the distension decreases, and later, when the piece becomes completely collapsed, the tentacles undergo atrophy (Child, '04*d*, pp. 207–212). The results of experiments of this kind on *C. æstuarii* are even more striking, for the atrophy occurs more rapidly. The records for two series of experiments are given by way of illustration.

Series 14.

August 30, 1905. — I. A piece with oral end about the middle of the œsophageal region (*a*, Fig. 3) and aboral end just distal to the aboral end of the œsophagus (*b*, Fig. 3).

II. As control a piece from another animal with oral end as nearly as possible at the same level as that of I., and with aboral end just proximal to the aboral end of the œsophagus (*c*, Fig. 3).

The difference in length of the pieces is very slight and both are far above the minimal size of pieces capable of complete restitution.

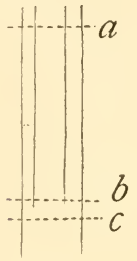
September 3. — I. Body-wall and œsophagus united at both

ends; piece somewhat distended with water; marginal tentacles just beginning to appear, about 1 mm. in length; no labial tentacles (Fig. 4).

II. Body-wall and œsophagus united orally so that a mouth is formed. Aborally the cut end of the body-wall is closed. The œsophagus therefore opens into the enteron. The piece is distended and the marginal tentacles are 2 mm. in length. No labial tentacles (Fig. 5).

September 9. — I. Piece slightly distended; marginal tentacles barely visible; no labial tentacles.

II. Fully distended; marginal tentacles 5–7 mm. in length; labial tentacles just visible.



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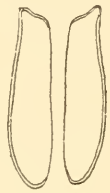
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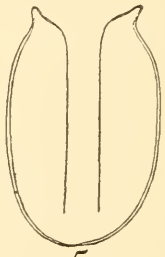
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September 18. — I. Collapsed; neither marginal nor labial tentacles visible.

II. Fully distended; marginal tentacles 8–10 mm. in length; labial tentacles 1–2 mm.

October 8. — I. Collapsed; much reduced in size and body-wall undergoing atrophy. No traces of tentacles.

II. In burrow, distended; marginal tentacles 18–20 mm.; labial tentacles 4–5 mm.

Later History. — I. After October 8 complete atrophy of the body-wall along the line of folds occurred and the piece broke up into fragments which continued to decrease in size and undergo atrophy until they died.

II. Remained in good condition in burrow until December 31 when experiment concluded. Marginal tentacles 20–25 mm. in length; labial tentacles 5 mm.

The difference in the process of regulation between these two pieces is very great. In piece I. the development of marginal

tentacles began during the period of temporary distention, but after collapse these tentacles atrophied and no trace of labial tentacles ever appeared. Moreover the piece gradually underwent decrease in size and atrophy and finally broke up and died.

Piece II., on the other hand, gave rise to a normal animal with both marginal and labial tentacles and remained in good condition for months.

Series 35.

September 15, 1905. — Four œsophageal pieces from different individuals, each including about the middle half of the œsophageal region.

September 20. — In all the œsophagus and body-wall have united at both ends and the enteron is without communication with the exterior. The pieces are slightly distended and one shows slight elevations of the body-wall in the marginal tentacle region; the others are without any trace of marginal tentacles, and none show any trace of labial tentacles.

October 2. — All collapsed and reduced in size. No traces of tentacles in any. In three pieces the body-wall has atrophied and split along the line of longitudinal folds.

Later History. — All four pieces gradually atrophied and broke up and the fragments died. No further traces of tentacles appeared at any time. During the same time other pieces of the same and smaller size but with aboral ends proximal to the œsophageal region, closed, became distended, gave rise to marginal and labial tentacles and remained in good condition until the conclusion of the experiment on December 31, 1905.

In general the development of marginal tentacles is almost completely inhibited in œsophageal pieces and the tentacles which do appear undergo complete atrophy later. Labial tentacles have never been seen in the pieces. Moreover the pieces are incapable of continued existence for more than a few weeks in the absence of internal pressure.

(*c*) *The Development of Tentacles on Oblique Oral Ends.*

In *C. solitarius* the formation of tentacles on oblique oral ends is not simultaneous about the whole margin as it is on transverse ends, but the tentacles appear earliest on the most distal portion

of the oblique surface and latest on the most proximal portion (Child, '04*d*, pp. 193–205, Figs. 1–6). At least a part of this difference is due to the difference in level of the most distal and most proximal portions of the oblique surface, but in the paper referred to it was shown that the marginal tentacles appear earlier on the most distal portion of an oblique end and later on the most proximal portion than on transverse ends at these two different levels. These and other results led me to suggest that perhaps the currents which pass orally along the inner surface of the body-wall in each interseptal chamber might, as well as the general internal pressure, be a factor in tentacle-development (Child, '04*d*, pp. 193–205).

The results of experiment on oblique pieces in *C. æstuarii* are similar : part of the records for one series is given here.

Series 33.

September 15, 1905. — I. The oral ends were removed from four specimens by oblique cuts through the œsophageal region (*bb*, Fig. 1).

II. The oral ends were removed from four other specimens by transverse cuts as nearly as possible at the same level as the most distal portion of the oblique cuts (*aa*, Fig. 1). No control was made for the lower level of the oblique surface because the rapidity of tentacle-formation on transverse surfaces is not very different at these two levels. The history of one of the oblique pieces and one of the controls is given.

September 17. — The cut ends in the oblique pieces I. and the control II. were closed : tentacles were not visible in either, but the body-wall was becoming thinner in the most distal portion of the oblique piece (Fig. 6, diagrammatic longitudinal section).

September 20. — I. Marginal tentacles about 2 mm. long on the most distal portion of the oblique surface and decreasing in length from this point to zero about half way between the extreme distal and proximal portions of the disc. The most proximal portion of the disc shows no preparation for tentacle-formation as yet. Fig. 7 is a diagrammatic longitudinal section and Fig. 8 shows one side of the disc.

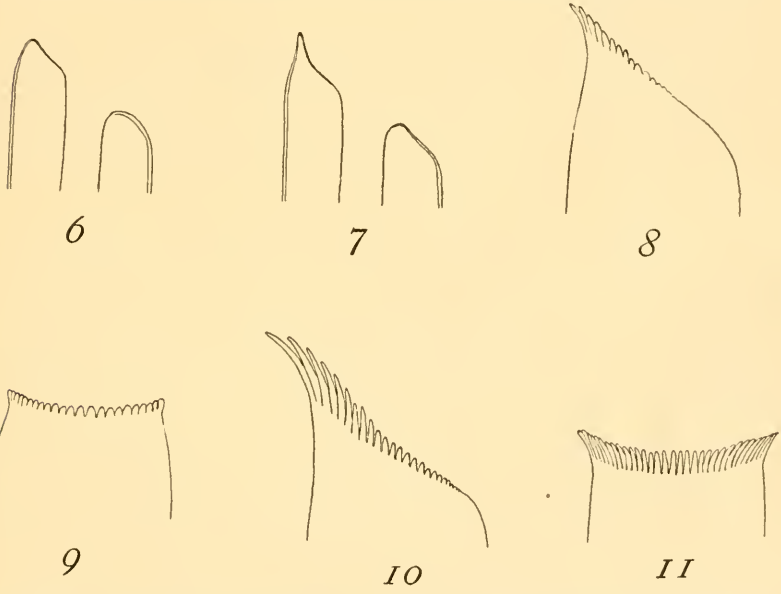
II. Marginal tentacles just appearing about the whole margin of the disc : 0.5–1 mm. in length (Fig. 9).

September 23. — I. Marginal tentacles on most distal portion 5–6 mm. in length, decreasing to slight elevations on the opposite side (Fig. 10).

II. Marginal tentacles 2–3 mm. in length about the whole margin (Fig. 11).

Later the tentacles on the oblique piece underwent a gradual equalization and the disc lost its obliquity as in oblique pieces of *C. solitarius*.

This series shows very clearly, and my other series afford similar results, that tentacle-formation is accelerated on the more

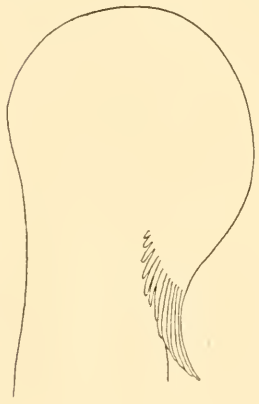


distal portions and retarded on the more proximal portions of the oblique piece as compared with transverse pieces.

Since internal pressure is in general so important a factor in tentacle-formation in *Cerianthus* it seems not at all improbable that the differences between the oblique and transverse pieces may be due to local differences in the internal pressure in the oblique pieces. The currents passing orally in each interseptal chamber enter the inrolled portion of the oral end and, as was suggested in my earlier paper, local differences in the internal pressure may be produced by these currents since the body-wall is always

folded over much more sharply on the more distal portions of the oblique end than on the more proximal. At all events the obliquity of the disc affects the rapidity of tentacle-formation in some manner, and the suggestion that local differences in internal pressure resulting from the different relation between the internal currents and the body-wall on different parts of the oblique end are concerned in producing the result observed seems at present to account for the facts. The question as to whether localized internal pressure is a factor in the localization of tentacles will be considered elsewhere in connection with certain experiments on another actinian.

One other series of experiments in which the plane of section of the body was very oblique deserves brief mention. In this series the plane of section extended from a region near the original tentacles on one side to a point considerably below the proximal end of the œsophagus on the other (*cc*, Fig. 1). The margin of the body-wall and the œsophagus united down to the proximal end of œsophagus, but since the cut extended a considerable distance beyond this point, there remained an opening of considerable size, which could be closed only by approximation of the margins of the body-wall and the formation of new tissue between them: this, however, occurred very slowly. The result of the operation was then at first a piece in which one side of the body-wall in the œsophageal region and to a point some distance proximal to it, and one side of the œsophagus were removed.



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The distal portions of these pieces underwent considerable decrease in length so that the obliquity of the oral end was reduced. In earlier stages distension with water was impossible because of the opening below the œsophagus, and tentacle-formation was much delayed. Later the most distal portion bent over and together with the slime secretion effected a provisional closure (Fig. 12), and some degree of distension took place and was fol-

lowed by the formation of tentacles. Below the œsophagus no tentacles formed because here no closure of the wound took place.

A month after the operation the pieces possessed the form shown in Fig. 12. The most distal part of the oral end was bent over so that the oblique disc was applied to the side of the body in the region of the opening below the œsophagus. The disc bore a semicircle of marginal tentacles which showed the usual differences in length of tentacles.

These pieces are of interest because they show an extreme type of reaction to the wound, but even in this case the reaction seems to consist essentially of contraction of the cut edges of the body-wall. The fact that this contraction produces so marked a change in the position of the oral end is due merely to the position of the cut.

III. Experimental Change in Length and Atrophy of Tentacles.

The marginal tentacles are 40–50 mm. in length when fully distended and the labial tentacles about 10 mm. Exact measurements are of course out of the question. Reduction and atrophy of the tentacles can be induced in this species as in *C. solitarius* by preventing distension of the body and tentacles. As has been noted above, openings in the body-wall do not entirely prevent distension, even when frequently reopened, for provisional closure occurs, sometimes within a few minutes after reopening. These provisional closures, however, cannot withstand anything like the normal internal pressure, so that even when they occur the total distension is very much below the normal.

In one series of this kind the tentacles were reduced in fifty days from full length to mere stumps, the marginal tentacles being 2 mm. in length and the labials just visible as slight elevations. Atrophy of the tentacles proceeds in the centripetal direction as in *C. solitarius*, and at the tip of each tentacle a small mass of degenerating tissue is visible (cf. Child, '04*c*, Figs. 3–5). If the specimens are allowed to close and become distended after atrophy of the tentacles, growth of the tentacles begins anew and proceeds according to the degree of distension established.

Tentacle-atrophy is a characteristic feature of pieces taken

entirely from the œsophageal region, in which the œsophagus and body-wall unite at both ends and there is no communication between the enteron and the exterior. The distension which occurs during the first few days after closure disappears later and complete collapse takes place. Under these conditions atrophy of the tentacles is very rapid: in some cases complete disappearance of fully developed normal tentacles occurred within thirty days. Tentacle-atrophy is always followed in these pieces by atrophy of the body-wall and finally fragmentation and death of the pieces.

Partial tentacle-atrophy can be induced in this species merely by keeping the animals in dishes without sand in which they can burrow. Without the support of the wall of the burrow the body-wall is unable to sustain the normal degree of internal pressure and the tentacles undergo partial atrophy in consequence of the altered conditions. Apparently the internal pressure is regulated to a greater or less extent by the strain upon the body-wall, for it is certain that in animals outside the burrows the internal pressure is not nearly as great as when the body-wall is supported by the wall of the burrow. So far as I can determine this difference is not due merely to escape of the water through the aboral pore when a certain degree of pressure is reached, for the pressure is always much below the point when opening of the aboral pore occurs: there seems rather to be a regulation of the entrance of water. Occasionally for some reason regulation does not occur or is insufficient and rupture of the body-wall takes place, usually in the aboral third, where the body-wall is thinner than in other regions.

After animals have been kept for five or six weeks under these conditions the marginal tentacles are usually 10–15 mm. and the labials about 5 mm. in length.

A marked difference in the length of tentacles produced in regulation likewise appears according as the animals are allowed to burrow or are kept without sand. Tentacles developing on pieces which live in burrows may attain the length of the original tentacles while those on pieces without sand reach only half this length or less.

The body-wall of *C. solitarius* is much thicker and more resist-

ant than that of *C. æstuarii* and is capable of supporting without the aid of the tube, a degree of internal pressure which closely approaches or perhaps equals the normal. In that species, therefore no marked reduction of the tentacles is observed in specimens kept without sand.

IV. Lateral Partial Discs.

The formation of lateral partial discs occurs in connection with lateral incisions and usually in the œsophageal region only, where the cut edges of the body-wall and the œsophagus fuse both above and below the cut and so give rise to a lateral mouth (Child, '05*a*). In all cases observed in *C. solitarius* the region between the lateral cut and the oral end of the body undergoes complete atrophy sooner or later and the lateral partial disc takes the place of the atrophied part. Atrophy occurs in this region because its enteric cavity has no connection either with the other portions of the enteron or with the exterior. After closure some distension occurs but collapse follows and continues until the part undergoes complete atrophy. Meanwhile the partial disc gradually changes its position toward the oral end and finally replaces the atrophied part.

(*a*) *Transverse Lateral Incisions.*

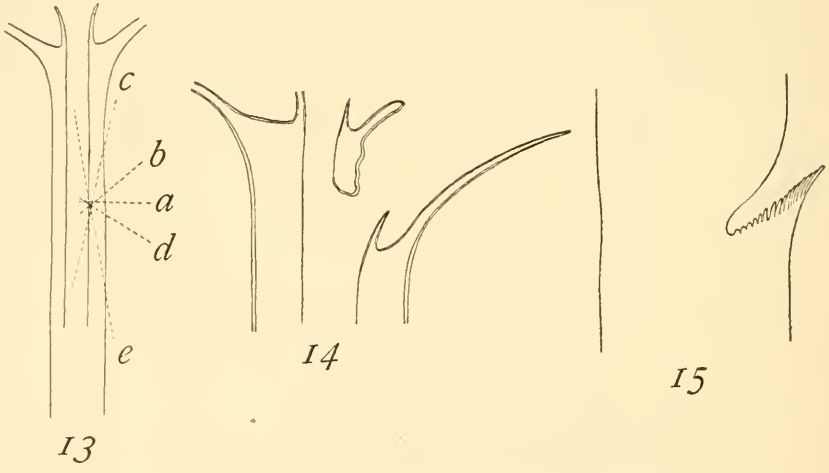
The only difference worthy of note in this connection between *C. solitarius* and *C. æstuarii* is the more rapid atrophy of the portion oral to the cut in the latter species. Frequently this atrophy is so rapid that breaks occur in the piece within a week or ten days after the operation, while in *C. solitarius* this condition is reached only after several weeks. Fig. 14 shows a longitudinal section of a specimen with lateral partial disc and the collapsed portion undergoing atrophy oral to it. The incision in this case was made at *a*, Fig. 13.

(*b*) *Oblique Lateral Incisions Directed Aborally.*

No oblique lateral incisions were made in my earlier work on *C. solitarius*, but a considerable number of operations of this kind have been made upon *C. æstuarii*. When the incision is directed aborally at any angle with the transverse plane up to 50°–60° (*b*, Fig. 13) partial discs appear which show the difference in

rapidity of tentacle-development of different parts of the disc characteristic of oblique discs (cf. pp. 35–38), the tentacles appearing earliest on the most distal portion and latest on the most proximal portion of the disc. A case of this sort is shown in Fig. 15. The later history of these oblique discs is similar to that of partial discs formed after transverse incisions, and during the change in position of the disc the obliquity gradually disappears as in other oblique discs and the result is an animal of usual shape.

But when the angle between the incision and the transverse plane is greater than 50°–60° (*c*, Fig. 13) the results differ from



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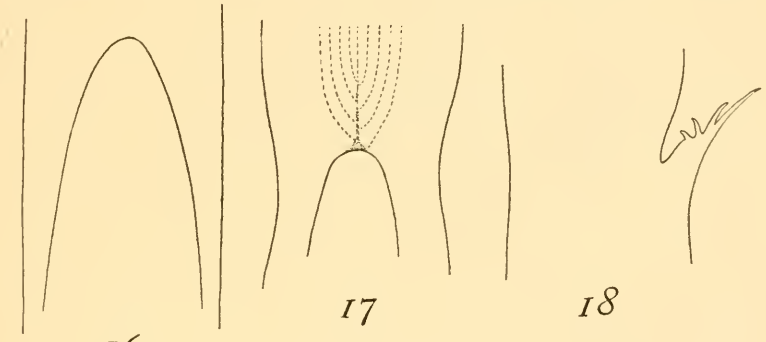
those described and in some cases permanent lateral discs are formed borne on a small column which arises as a branch from the side of the original body. As the plane of the incision approaches the longitudinal axis of the body the incision approaches a longitudinal direction and the process of wound-closure approaches that occurring after a longitudinal incision. The course of an oblique incision approaching the longitudinal direction is indicated in Fig. 16. After an incision of this kind the wound in the body-wall closes longitudinally by approximation and union of the cut margins (Fig. 17), but the oblique slip of tissue which was separated distally from the body-wall but is still connected with it proximally is not included in the closure and its cut margins also close longitudinally and independently of the

other part except at its proximal end where it is continuous with the rest of the body-wall. Thus the closure of the wound results in the formation of a small component extending obliquely in the distal direction from the side of the body. The œsophagus also closes longitudinally in each part and in the lateral component unites distally with the body-wall so that this component possesses a mouth. In this manner an oblique lateral disc is established at the distal end of the lateral component, but it is a complete not a partial disc, because the closure has been largely longitudinal. On such discs the tentacles arise in the manner characteristic for oblique discs, *i. e.*, earliest on the most distal portion and latest on the proximal portion of the disc (Fig. 18).



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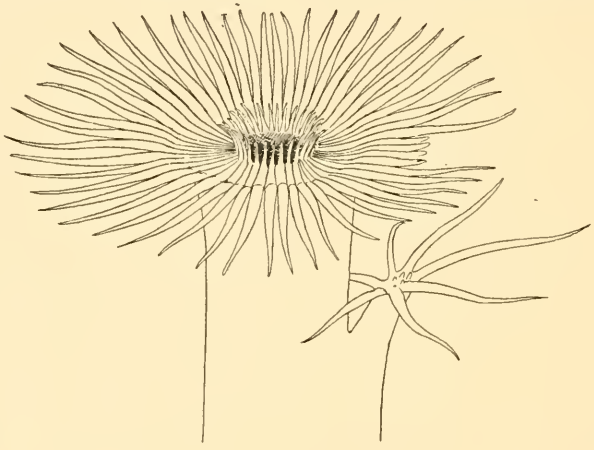
In Fig. 19 one of these cases is shown after complete development of the tentacles on the lateral disc, but before the obliquity has disappeared. In this figure it is seen that several of the tentacles on the original terminal disc, viz., some of those which were situated directly distal to the incision, have undergone partial atrophy. The stage figured does not, however, represent the greatest degree of atrophy which occurred. After the closure of the wound these tentacles underwent rapid atrophy until they were reduced to less than half the length shown in the figure and some of them were mere stumps 2–3 mm. in length. Then they began to grow again and would undoubtedly have finally attained the same length as the others if it had been possible to continue observation of the specimen for a longer time. The reason for the peculiar history of these tentacles is to be found in the method of closure of the wound. In Fig. 17 the direc-

tion of the septa in the region of the incision is indicated diagramatically by the dotted lines. With the approximation of the oblique margins of the cut the cut ends of the septa are also closely approximated, and since the œsophagus closes in the same manner as the body-wall the consequence is that some of the interseptal chambers distal to the apical region of the cut become almost or completely closed aborally, while lower down, where more new tissue is formed between the old margins of the body-wall they remain more widely open. When the body is distended with water those chambers which are nearly or quite closed aborally remain almost or wholly collapsed, since they



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are nearly or quite isolated from the other parts of the enteron, and the pressure resulting from the more rapid distension of other parts aids still further in closing small openings which may remain. After the distension resulting from the passage of water through the body-wall or septa has subsided this region remains collapsed and the tentacles corresponding to those chambers begin to atrophy. But in the further course of regulation after closure the area of new tissue increases and the old margins of the cut, now united by a thin membrane of new tissue, gradually separate as it increases, and the relations of the septa also undergo regulation in consequence. At a certain stage the interseptal chambers which were shut off from other parts of the

enteric cavity again become open and fill with water and renewed distension of the tentacles and consequently renewed growth occurs.

But the most interesting feature of these cases is that, so far as my observations go, the lateral component is permanent and does not undergo further regulation ending in union with the parts of the terminal disc which remain, as do the transverse and less oblique lateral discs. Some specimens were kept under observation during four months and at the end of this time there was no indication of any further regulation leading to fusion of the lateral component with the rest of the body. Fig. 19 is drawn from one of these specimens four months after the operation. This length of time is more than sufficient for the migration of the lateral disc to the oral end of the whole if it were to occur, but there is no evidence of any such change in position. Each component maintains its form and relations to the other. The only changes noted in later stages were, the increase in length of the lateral component, which would probably in time have reached the level of the original disc, the gradual reduction of the obliquity of the disc of the lateral component and new growth of the previously atrophied old tentacles above the cut.

The reason for the failure of the lateral disc to migrate to the oral end of the body and take the place of a part of the original disc lies in the simple fact that no part of the region distal to the cut underwent complete atrophy. Atrophy began, but the regulatory process involved in the filling of the area of the cut with new tissue brought about the opening and renewed distension of the interseptal chambers in this region and so determined its persistence. Evidently the presence of active non-atrophied parts distal to the lateral component prevents its migration to the oral end.

These cases are of considerable interest in that they indicate very clearly the dependence of "normal" proportions and positions of parts upon certain factors which are subject to experimental control, viz., in this case the direction of the incision and the distension or absence of distension of certain parts with water and their consequent persistence or atrophy.

(c) Oblique Lateral Incisions Directed Orally.

When the oblique incision is directed orally the results differ, according to the angle as in the case of aborally directed incisions described in the preceding section. If the angle between the incision and the transverse plane is not greater than 30–40° (*d*, Fig. 13) a lateral partial disc with mouth is usually formed, the parts distal to it atrophy and it gradually migrates to the oral end of the body. The sequence of tentacle-formation in these partial discs is that characteristic of other oblique discs, the tentacles appearing earliest on the two opposite ends of the partial disc, which in these cases are the most distal portions, and latest on the middle, most proximal portion.

When the orally directed incision is more oblique, however (*e*, Fig. 13) other results are obtained. In most cases of this sort complete closure of the wound occurred sooner or later without the formation of a lateral mouth or partial disc, *i. e.*, the cut margins of the body-wall united longitudinally with each other and not with the œsophagus, and the oblique slip, which in these cases is directed aborally, formed a small shrivelled excrescence, which underwent gradual atrophy and resorption.

After these incisions the margins of the body-wall approximate and unite longitudinally from the proximal end of the cut distally (Fig. 20, the area of new tissue is indicated by shading). When the approximation of the margins and the accumulation of slime has brought about a provisional closure and partial distension is possible, the more distal portions of the cut margin are pressed against the aborally directed slip which overlaps this region and union readily occurs here, so that the whole wound is closed without the formation of a disc. Here the result, in itself different from that obtained with aborally directed oblique incisions, is brought about by the same internal factors, *i. e.*, the characteristic reactions of the species, but the conditions under which the reactions occur are different, hence the difference in result. In the case of aborally directed incisions the partial distension separates the oblique slip from the rest of the body-wall, since the slip shares more fully in the distension than the portion of the body-wall directly distal to the cut. In the present case, on the other hand, the oblique slip is less distended than other

parts, hence it contracts and the other parts are pressed against it, permitting union of the cut margins to occur.

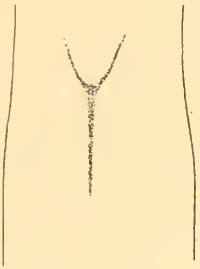
In several cases of this sort the incisions were reopened one or more times after they had healed in the manner above described, a .. in one case tentacles were produced, but later disappeared. The history of this case is briefly given since it presents some features of importance.

Series 31—III.

September 1. — Very oblique incision directed orally.

September 9. — Almost entirely healed; reopened.

Marginal tentacles on original disc 12–15 mm. in length except



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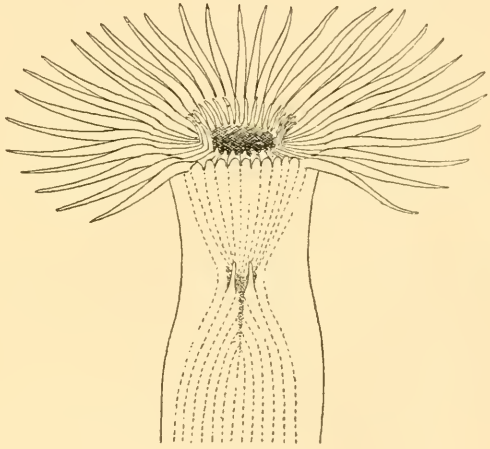

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above cut, where they are reduced to about 1 mm. Labial tentacles 3–4 mm., except over cut, where 1–2 mm.

September 28. — (Fig. 21.) Marginal tentacles on original disc 10–12 mm. in length except over cut, where they are reduced to about 1 mm. Labial tentacles 2–3 mm., except over cut, where they are scarcely visible.

The lateral wound is closed by thin new tissue, but near its distal end two small tentacles appear, one arising from each side of the cut. Proximal to the tentacles the cut margins have united longitudinally and distal to the tentacles a broader area of new tissue fills in the space between the old margins. The course of the septa is indicated by the dotted lines in Fig. 21. It is evident

that tentacles could not arise proximal to the two which have appeared, because there the two sides of the cut united almost directly. The two tentacles represent the last interseptal chamber on each side which was opened by the incision. The next interseptal chambers lateral to these on each side are continuous to the oral end of the body and the first of the normal tentacles on each side above the cut corresponds to one of them. The reason for the atrophy of the old tentacles above the cut is at once apparent from the figure. In consequence of the approximation of the sides of the cut and the contraction of the oblique slip the interseptal chambers to which these tentacles belong have been cut off from communication with the rest of the enteron. In the living specimen this portion of the body was very evidently collapsed, while other parts were distended.

October 11. — No marked changes since September 28.

December 31. — During the period between October 11 and this date the specimen was not examined closely since the formation of tentacles in the lateral region was the chief purpose of the experiment. On this date, when the experiment was concluded, examination showed that the two small tentacles had disappeared, the new tissue which closed the wound had become thicker and more like the old body-wall and extended over a larger area than before, and finally the tentacles above the cut on the terminal disc had once more grown out to the same length as the others. At this time the marginal tentacles were 7–8 mm. in length and the labials 1–2 mm. In the course of regulation the contracted area resulting from the approximation of the margins of the cut had again spread out in consequence of the growth of new tissue and the interseptal chambers were again in communication with the rest of the enteron, hence the tentacles corresponding to them, which were previously atrophied, had developed to the same length as the others.

During a considerable part of this time the animal was in a burrow which it had made for itself in the sand, and it is probable that the pressure of the body-wall against the wall of the burrow aided in bringing about the disappearance of the two tentacles.

In this case, as in those discussed above, the special result is determined by the conditions of the experiment. No new method

of regulation is involved, but merely the same complex of reactions as in other cases under different conditions.

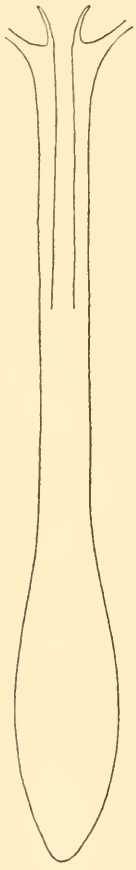
From these experiments with transverse and oblique lateral incisions it is evident that the formation of a lateral partial disc (Figs. 14 and 15), a lateral complete disc borne on a column arising like a lateral branch from the side of the body (Fig. 19), the formation of a few tentacles which disappear later (Fig. 21), or the closure of the wound without formation of a disc may each be determined by the conditions of the experiment. These different results cannot be interpreted as in any sense purposive or adaptive responses of the animal to the special conditions, since the nature of the reactions themselves is not altered, but merely their position, relation and sequence, and these are very clearly determined by the conditions of the experiment.

The Effect of Internal Pressure and its Absence upon the Body-Wall in General.

As was noted above, the body-wall of this species is thinner and more delicate than that of *C. solitarius* and very much more so than that of *C. membranaceus*. It is also much more sensitive and reacts more rapidly to the mechanical conditions resulting from internal pressure than do the tissues of either of those species.

Pieces of *C. solitarius* cut in such manner that closure is impossible remain alive for months, though they undergo gradual decrease in size and atrophy and finally break up and disintegrate. Pieces of *C. æstuarii*, on the other hand, in which no distension is possible, atrophy much more rapidly. The atrophy begins at points where the body-wall is sharply folded, and splits and breaks often occur in such regions within a week or ten days after the operation. The whole piece often breaks up and disintegrates completely in the course of three or four weeks — sometimes even more rapidly. Particular regions of the body-wall which remain collapsed for any considerable length of time undergo complete degeneration and disintegration. In short, it is evident that an essential condition, not only for the formation of new substance, but for the continued existence of the body-wall and tentacles in this species is the mechanical condition resulting from distension of the enteron with fluid.

On the other hand, the body-wall of this species is incapable of supporting alone the pressure of the enteric fluid. Under normal conditions the body-wall is supported by the wall of the burrow in which the animal lives and so does not support the entire pressure. When the animals are kept without sand in



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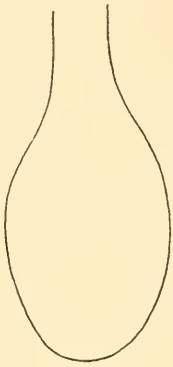

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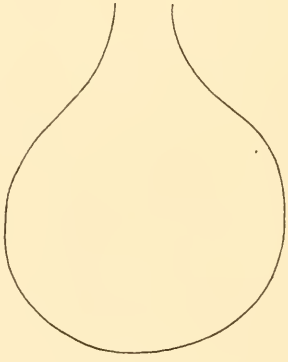

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which to burrow the shape of the body undergoes marked changes, especially at the aboral end, where the wall is thinnest. Under these conditions the aboral region of the body gradually increases in diameter and decreases in length (Fig. 22) and the change involves more and more of the body (Fig. 23), until in some cases almost the whole body aboral to the œsophagus approaches a

spherical form (Fig. 24). Such specimens are absolutely incapable of burrowing and never regain their usual form, at least no return was observed during a period of something over four months. Frequently, however, the change in shape ceases before the extreme condition is reached, *e. g.*, at a stage resembling Fig. 23 and the animal retains this shape afterward. The cessation of the change in shape appears to be a functional reaction of the tissues to the altered conditions to which they are subjected, *i. e.*, the increased tension in the body-wall develops increased resisting power, a change similar to that occurring in many other so-called functional adaptations. On the other hand, rupture of the body-wall frequently occurs in the enlarged region. These facts demonstrate that the tubicolous habit is an important factor in determining the shape of the body and the functional character of the body-wall.

VI. Discussion and Summary.

C. æstuarii demonstrates even more clearly than *C. solitarius* the importance of internal water-pressure for regulatory development and for the continued existence of the body-wall and tentacles.

On the other hand, there is no evidence that changes in the turgor of the cells themselves play an important or definite rôle in form-regulation, except that atrophy appears to be accompanied by a decrease in the turgor of the tissues involved. There is certainly no appreciable change in the turgor of the cell such as Moszkowski ('07, p. 412) describes for *Actinia* and *Actinoloba*. If Moszkowski's observations are correct, and certainly observations on *Cerianthus* do not permit conclusions concerning other forms, they simply afford another illustration of the physiological differences which may exist in different species.

The passage of water through the body-wall and the consequent distension of the body occurs in *Cerianthus*, as noted above and in my earlier papers (Child, '04*b*, pp. 276–277), as well as in *Actinia* and *Actinoloba* (Moszkowski, '07, p. 412), but it has not been possible thus far to discover any indications of a marked change in turgor of the cells themselves, and the results of my experiments show very clearly that the water in the enteron is an essential factor in form-regulation in *Cerianthus*.

According to Moszkowski ('07, p. 420), the decreasing rapidity of regulation with increasing distance from the oral end of the body is due in *Actinia æquina* merely to the fact that below a certain level a new œsophagus is formed and the parts of the old œsophagus which remain hinder closure until they are cast off.

In *Cerianthus* this is not the case. In all of the observed cases any portion of the œsophagus which remains takes part in the process of regulation and is not cast off. Moreover, differences in the rapidity of regulation are distinguishable both at different levels within the œsophageal region and at different levels aboral to it. It is to be expected that any difference in capacity which might exist at different levels of the body would be more marked in the greatly elongated body of *Cerianthus* than in such a form as *Actinia*, where the body is relatively short. At any rate, there is no doubt that such a difference exists in very considerable degree in *Cerianthus*.

The most important results of my experiments with *C. æstuarii* are stated in the following summary.

1. In *C. æstuarii*, as in *C. solitarius*, the distension by water in the enteric cavity is an essential factor in form-regulation. In its partial or total absence the formation of disc and tentacles is retarded or inhibited.

2. The internal pressure is essential not only for the formation of new parts, but for the persistence of the old. Partial or total atrophy of the tentacles follows decrease or absence of distension and the atrophied structures develop anew when distension is again permitted to occur.

3. The body-wall of *C. æstuarii* is much thinner and more delicate than that of *C. solitarius* and is also much more sensitive to changes in internal pressure. In the absence of distension even the body-wall undergoes rapid atrophy and disintegration.

4. In nature the walls of the burrow in which the animal lives aid the body-wall in supporting the pressure resulting from distension, especially in the aboral region. If the animals are kept in water without sand in which to burrow, the internal pressure never reaches its normal amount. Under these conditions the tentacles are more or less relaxed and undergo partial atrophy and the aboral region of the body becomes greatly deformed and

often ruptures because of its inability to sustain the existing pressure. In some specimens a "functional adaptation" to the altered conditions occurs and the body-wall gradually acquires the strength necessary to support the pressure. In such cases the partially atrophied tentacles may increase in length but in no case observed did they attain the length of tentacles of specimens living in burrows. Regulatory tentacles likewise fail to attain full length when the specimens are kept without sand, but do attain full length when they are permitted to burrow.

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THE DEVELOPMENT OF THE NUCLEI OF THE SPINNING-GLAND CELLS OF PLATYPHYLAX DESIGNATUS WALKER (TRICHOPTERON).

C. T. VORHIES.

The large and complexly branched nuclei of the spinning-gland cells of trichoptera (and lepidoptera) contain two kinds of stainable material. That one kind of this material is chromatin there is no doubt; that the other is nucleolar material perhaps the majority of cytologists now believe, but there have been some differences of opinion concerning this point. These materials in the glands of trichoptera — at least in *P. designatus* and some other species — consist of larger, irregularly shaped masses, and small, more evenly sized granules, which have a roughened appearance as if made up of smaller particles. In the lepidopteron, *Isia isabella*, the two materials are scarcely distinguishable by the size of particles, but only by their staining reactions. The smaller granules stain blue in the triple stain of Flemming, while the larger take the red stain characteristic of nucleolar material. These larger particles will be hereinafter referred to as nucleoles or nucleolar material.

A brief review of the literature of this subject seems desirable.

Helm (1876), though he indicates the ontogenetic changes of form of the nuclei, does not distinguish the contents of the same.

Carnoy (1884), in a rather diagrammatic drawing (Fig. 54) of a spinning-gland cell from the larva of a trichopter, shows filaments of "nucléine" which undoubtedly represent the nucleolar material. He does not distinguish the chromatin in this drawing. In Figs. 78 and 79 he shows a portion of a branched nucleus from the gland of a moth larva, and a cell from the gland of the larva of a microlepidopter. In both of these, the nucleolar material and chromatin are indicated, but the chromatin is not mentioned and the nucleolar material is designated as the "boyau nucléinien." This skein of nuclein he believes to be

made up in its turn as in a nucleus — a quite incorrect view, of course, as we know it. In his Fig. 132, *A* and *B*, he indicates the difference in form of the nuclei from young and old larvæ of a microlepidopter.

Gilson (1890) figures sections of spinning-gland nuclei of lepidoptera, but does not distinguish the character of the nuclear contents, nor does he discuss the nature of the material. The same author (1894) distinguishes the two materials in glands of trichoptera. He mentions the parts as follows: "Disons seulement qu'il existe au sein de l'amas de tronçons nucléiniens, des corps arrondis, chromatique, des nucléoles particuliers qui souvent laissent voir dans leurs interieure des cordons nucléiniens, semblables à ceux qui constituent la grande masse du contenu nucléaire."

Korschelt (1896), working on various species of lepidoptera, used a modification of the Ehrlich-Biondi stain. He found the larger particles (macrosomes) to stain green, the smaller ones (microsomes) to stain red, and concluded that the macrosomes must be regarded as chromatin, the microsomes as nucleoli.

Meves (1897), employing various methods of staining, shows that the microsomes of Korschelt are chromatin granules, and that the macrosomes of that author must be regarded as nucleoli. He employed Heidenhain's formula of the Ehrlich-Biondi stain and got the opposite result to that of Korschelt, who used a formula with the methyl green much stronger.

Korschelt (1897) upholds the view previously expressed by him as to the nature of the macrosomes and microsomes.

Flemming (1897) agrees with Meves.

Henneguy (1904), p. 463, refers to the conclusions of Korschelt and Meves and records his researches as agreeing with the latter.

Marshall and Vorhies (1906) conclude as the result of the use of various stains, that the larger bodies are nucleoles, the smaller granules chromatin.

It is evident from the above review that all of our evidence upon the nature of the stainable materials is that derived from the staining reactions. While with our present knowledge of such reactions the proof thus offered is very good, yet the presence of such large amounts of nucleolar material as are found in the

spinning-gland nuclei and the fact, as shown by Korschelt's work, that changes in the formulæ of certain stains may give conflicting results, it seems very desirable that evidence of a somewhat different kind be obtained, if possible. It was with the object of ontogenetic evidence in view that the present work was begun.

It may be remarked at the outset that it was not anticipated that the task would prove quite so easy of accomplishment as it has, since it was supposed that the later embryonic stages would require investigation. This proved not to be the case, however, the earliest stages necessary to solve the problem being the young larvæ as they emerged from the egg.

Methods. — It was found that the spinning-glands must be dissected or teased out before fixation to secure the best results, since even the youngest larvæ are not penetrated readily by the fluids. Decapitation gives quite good results, as some parts of the glands will then usually protrude from the cut end of the body. The most satisfactory method found for the smallest larvæ was to plunge them alive into the fixative, then turning the ventral side up, and holding the posterior end down with a needle, the head can be caught with the point of another needle and pulled off. The glands, not being fastened posteriorly, will in most cases draw cleanly out of the body. With larger larvæ this is not easy of accomplishment, and decapitation is the better method. With the largest larvæ, dissection from the dorsal side while immersed in the fixative is best. Flemming's strong formula, Tower's solution and 95 per cent. alcohol were most used, as fixing agents. As described in previous papers, Vorhies (1905), Marshall and Vorhies (1906), Delafield's hæmatoxylin was most satisfactory for whole mounts of the glands. The method of splitting the glands previously described cannot, of course, be used with the glands from larvæ only 1.5 mm. to 2 mm. in length, but the difficulties to be overcome by splitting in the larger glands are in these not so great, therefore it does not matter. For sections, Flemming's triple stain, and iron hæmatoxylin were used almost exclusively. Both sections and whole mounts were made of the various stages, the whole mounts serving as a useful check on the sections, and to show the changes in form of the nuclei.

At the time of hatching the larvæ of *P. designatus* are about

1.5 mm. in length. Glands from such larvæ show very clearly the two rows of cells forming the wall of the gland, and the addition of a little dilute methyl green to a drop of normal salt solution containing them shows at once that the nuclei are of a simple, unbranched type: typically the nuclei are round at this stage, though a few are slightly elongated. It may be noted here that from the time of breaking out of the egg membrane to the time of emergence from the mass of jelly containing the eggs may be some hours for an individual, and 24 hours barely suffices for a brood to get clear of the jelly after the first ones are seen moving within the mass. To distinguish carefully in every case between those larvæ fresh from the egg membrane and those just out of the jelly would be rather more laborious than results would warrant, so in speaking of larvæ just hatched I mean larvæ in the jelly or out of it only a few hours.

In each of the round or slightly elongated nuclei of this first period there is, almost without exception, a single nucleole present (Figs. 1 and 2). This nucleole is large, round or elongated, smooth in outline, lies near the center of the nucleus, takes the stains characteristic of the nucleoles of ordinary cells, and, in short, is undoubtedly a true nucleolus. A study of fresh glands of larvæ just out of the egg or even yet within it, shows that the elongated (rarely divided) nucleoles belong generally to the larvæ which have been for some time out of the egg.

Larvæ which have been out of the jelly for twenty-four hours, and which have been supplied with sand, possess a well constructed case. Glands from such larvæ contain a larger proportion of elongated nuclei, the elongation being transverse to the long axis of the gland. In whole preparations there may be found single round or elongated nucleoles, and, in many instances, two or three nucleoles in one nucleus (Figs. 3, 5, 6). Sections at this stage show that the two nucleoles in a nucleus arise *by division of the original one*, since various stages of elongation and constriction, giving more or less dumb-bell-shaped figures may be found. By division here is not meant bipartition necessarily, but fragmentation, since evidences of the latter process are readily observable (Fig. 4). From this time on the nucleoles are more ragged and irregular in appearance, but this may

be due to the metabolic activity of the gland (Marshall and Vorhies, 1906).

On account of the difficulty of keeping the larvæ in the laboratory the exact ages of the larvæ with reference to the following events have not been obtained, but that is scarcely necessary for the purpose of this work. With glands from larger and larger larvæ, more and more of the nuclei are found to contain two nucleoles, occasionally three or four are present (Figs. 7, 8), and in larvæ about one week old, and 2 mm. or more in length (which may have molted) as many as 4–7 nucleoles are typically present in each nucleus (Figs. 8, 9). The nucleoles now increase in number continuously as the nuclei increase in size (Figs. 10–14), and, except that they become somewhat more uneven in size and more irregular in shape, there is little to be noted. A more detailed account with drawings showing their characteristics in various large nuclei is contained in the 1906 paper already referred to.

The changes in form of the nuclei, already briefly mentioned, consist first in a lengthening in the direction of the circumference of the gland (Figs. 2, 4–7); in a larva one week old, the nuclei are two or three times as long as they are wide (Figs. 7–9). It will be noted that there is a marked increase in number of nucleoles before there is any great change in shape of the nuclei (Figs. 9, 10). Swellings next appear, which elongate and develop into branches (Fig. 11). Since there are no distinct centers of branching (Vorhies, 1905), as figured by Henneguy (1904), there is no regular order of development to be traced. At first, it appears that a majority of the elongated nuclei have two branches at one end forming a T-figure, or two at each end, forming a kind of modified H-figure (Fig. 12), but if there really is any such tendency the increasing complexity of the branch system soon obscures it. The complexity simply increases with the increase in size of the cell (Figs. 13, 14). There does not, however, appear to be a high degree of correlation between the size of the cell and the space occupied within it by the nucleus (Marshall and Vorhies, 1906, Figs. 1–6).

The condition of the chromatin remains the same, so far as its staining reaction and appearance are concerned, throughout the

nuclear history as outlined. The granules in the large nuclei are of about the same actual size, with the same inter-granular spaces, as in the young rounded nuclei. No evidence was noted in the staining reaction which might lead to the conclusion that those of the younger nuclei were merely more dense. Indeed, the difference in size of the nuclei is so great that it is almost impossible to conceive of the chromatin of one of the large nuclei being compressed or condensed to the relatively few granules found in a small one. The chromatin must therefore increase in amount. In all stages a linin reticulum is easily distinguishable with the higher magnifications, particularly in sections.

Conclusions.

1. The larger particles of stainable material in the spinning-gland nuclei of *P. designatus* are derived by division and growth from an original nucleole of normal type and hence may be regarded as true nucleoles.

2. The red-staining granules in the nuclei of similar glands of lepidoptera, whether larger than the chromatin granules or not, are probably of similar origin and character.

3. This nucleolar material increases in amount with the growth of the nucleus.

4. The latter conclusion coupled with one in a former paper (Marshall and Vorhies, 1906, p. 417), that the nucleoles become irregular as a result of glandular activity, leads to the further conclusion that the nucleolar material bears a direct relation to such activity: whether as a waste product or as a material functional in secretion I make no assertion.[1]

5. The chromatin increases in amount with the growth of the

[1] In connection with conclusion 4, attention may be called to the following from Montgomery (1899), p. 537. "The hypothesis might be suggested that though the nucleolus probably consists of substances which stand in some relation to the nutritive processes of the nucleus, and so at the time of its formation may be a functionless, inert mass of substance, yet it may at later periods in the history of the resting nucleus acquire some active function and thus gradually come to acquire the value of a nuclear organ; this hypothesis is put forward merely as a tentative one. According to this view the nucleolus might be considered as an organ which serves to accumulate in itself the waste products of the nucleus, thus serving as a reservoir for such substances; or it might be considered as an organ of excretion, to discharge waste products out of the nucleus; in either case the nucleolus would seem to stand in direct connection with the nutritive substances and forces of the nucleus."

nucleus, and therefore probably has a functional part in the process of secretion. The interesting question now arises as to whether the material which thus increases for this purpose is identical with the material which we believe to be the bearer of the hereditary qualities, or whether it is of a different nature functionally, but with the same staining reaction. This must remain an open question. There seems to the writer no reason why the same material cannot determine the direction of development of the cell (*i. e.*, carry hereditary qualities) and also determine the functional activity of the cell after its differentiation.

In connection with the last two conclusions it may be well to note that it has been repeatedly stated that in the process of yolk formation in eggs, cytoplasm (yolk) is formed from substance which is given off from the nucleus as buds (Blochmann, Scharff, Balbiani), as extrusions of parts of the chromatin (Fol, Blochmann, Van Bambeke, Erlanger, Mertens, Calkins), or as nucleolar substance (Leydig, Balbiani, Will, Henneguy): also that Miss Huie (1897) working on gland cells of *Drosera*, and Mathews (1899) working on pancreas cells, got analogous results with those secretory cells.

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Explanation of Plate I.

All nuclei figured which are longer in one dimension than in the other lie with the long axis transverse to the long axis of the gland.

Fig. 1. Nucleus of a spinning-gland cell from a larva just hatched; the single nucleolus is typical in size, shape and position. × 1,300.

Fig. 2. A nucleus from the same gland as that in Fig. 1. Nucleolus elongated. × 1,300.

Fig. 3. Nucleus from a larva "just hatched" but probably a little older than the one from which Figs. 1 and 2 are taken. × 1,300.

Fig. 4. Nucleus from the same larva as Fig. 3. Nucleole fragmenting. × 1,300.

Fig. 5. Slightly elongated nucleus from same larva as Figs. 3 and 4. Two nucleoles. × 1,300.

Fig. 6. Slightly elongated nucleus from the same larva as Figs. 3, 4 and 5. Two irregular nucleoles. × 1,300.

Fig. 7. Elongated nucleus from a larva twenty-four hours old. Nucleoles irregular, three in number. × 1,300.

Figs. 8 and 9. Larger nuclei from a larva about one week old, showing further fragmentation of the nucleolus. × 1,300.

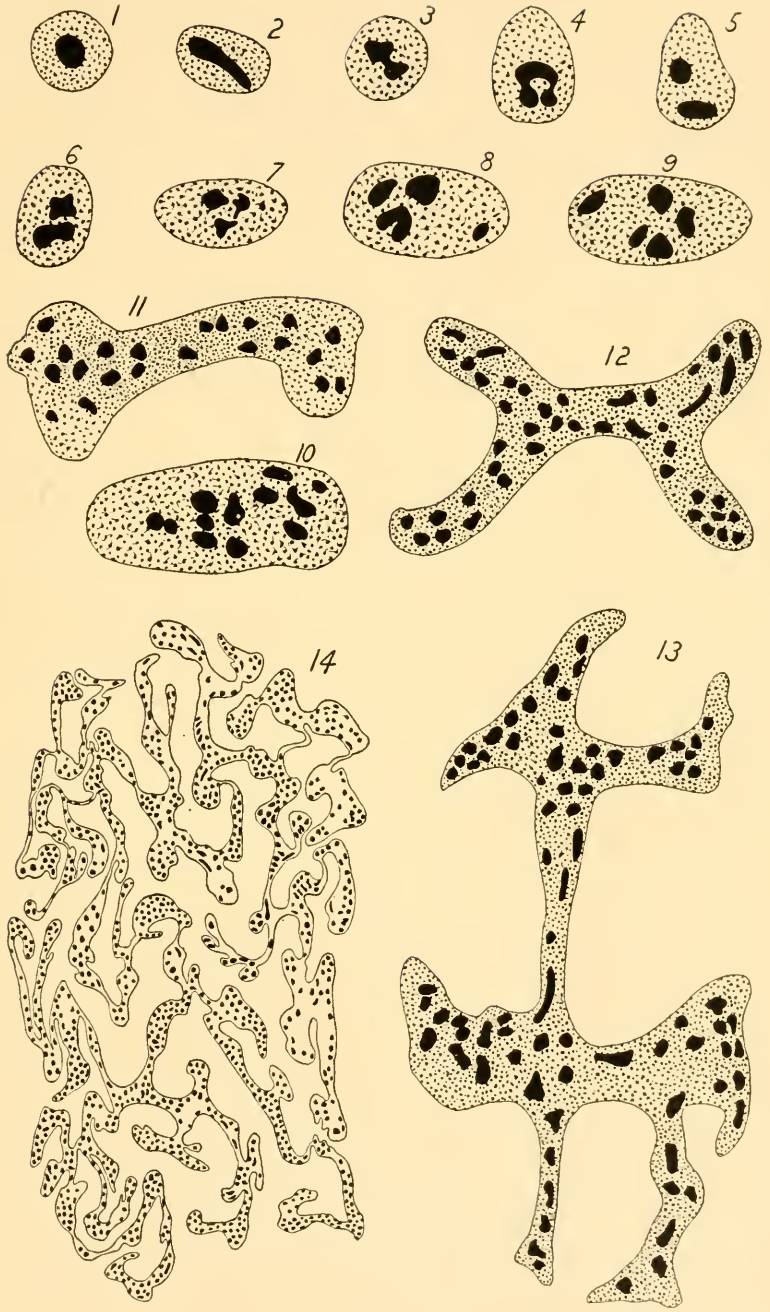
Fig. 10. Nucleus from a larva about one week old, which has probably molted. Twelve nucleoles present. × 1,300.

Fig. 11. Nucleus from a larva about 3 mm. in length. It is probably about two weeks old and has certainly molted. Twenty-two nucleoles. × 800.

Fig. 12. Nucleus from another larva 3 mm. in length. Many nucleoles. Ends of the nucleus branching. × 800.

Fig. 13. Nucleus from a larva 5 mm. in length. Branching more complex, and nucleoles more numerous. × 800.

Fig. 14. Nucleus from an adult larva, about 16 mm. in length, containing a very large number of nucleoles. No attempt has been made to represent the chromatin in this figure, as the magnification is not great enough. × 175.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THE OVOGENESIS OF HYDRA FUSCA — A PRELIMINARY PAPER.

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Kleinenberg in his thesis on hydra, 1872, maintained the following twelve points in regard to the formation of the ovary and the growth of the egg :

1. The ovary is formed by a rapid multiplication of the interstitial cells at the site of the ovary.

2. There is evidence in the relative infrequency of the interstitial cells, in the territory about the forming ovary, that their accumulation may be, in part, due to migration, though he has no direct evidence of this.

3. The interstitial cells at the center of the ovary are larger than those at the margin.

4. The interstitial cells are arranged in rows converging toward the center of the ovary.

5. The egg appears in the midst of the cells of the ovary where it has likely lain, indistinguishable from the interstitials.

6. It now comes to be distinct because of its rapidly increasing size and its irregular contour.

7. Later it becomes amœboid.

8. In the early stages of the egg granules appear in its substance which are equivalent to the white of the egg (protagon) of the eggs of higher forms.

9. These disappear and then the egg gradually fills up with the so-called " Pseudozellen " or yolk granules.

10. The cells about the egg in the ovary disintegrate to form nutritive material for the egg.

11. The egg thus increases greatly in size and with the mass of interstitials about it, greatly distends the ectoderm cells which elongate into fibers and are crowded to one side.

12. The egg breaks through these restraining ectoderm cells which are transformed into lamellæ at their peripheral end but are still connected with the muscle layer of the mesoglœa.

13. Brauer [1] describes the newly extruded egg as spherical, except for a stalk which attaches the egg to the parent. There is, too, a conical depression in the peripheral layer of the egg which layer is free from yolk. This depression lies opposite the attaching stalk over the nucleus, and by it the sperm has access.

14. Brauer states that the hydra egg gives off two polar bodies.

In attempting to confirm these results on sections of hydra prepared while working on another hydra problem, evidence accumulated that seemed to contradict some of these results. Additional material now convinces me that some of these statements are incorrect for *H. fusca* and permits me also to settle some additional points, notably on the chemical nature and the exact method of the inclusion of the yolk.

1. The multiplication of interstitial cells at the site of the ovary is by mitosis, twelve chromosomes appearing in the figure.

2. The interstitials are no less frequent immediately about the ovary than elsewhere, so there is no evidence of their migration into the region of the ovary.

3. The cells at the center of the ovary are larger than those at the margin, as Kleinenberg states. My measurements show that the central cells average four times, or a trifle more, the volume of the marginal. It is to be remembered that these central cells are adjacent to the growing eggs. The influences causing growth seem to operate on the whole region.

4. Kleinenberg, working largely on *H. viridis*, and later authors, notably R. Hertwig, working on *H. fusca*, claim that the egg appears only after the ovary has achieved considerable size. My sections seem to force the conclusion, however, that the egg is always present, before proliferation of the interstitials begins to form the ovary. The egg is often, and so far as I can see always, growing rapidly before the interstitials begin to multiply. It

[1] *Zeit. f. wiss. Zool.*, V., 52, pp. 167–216.

looks, therefore, as if the increase in size of the egg or eggs might be the cause or at least the occasion of the multiplication of these insterstitial cells.

5. R. Hertwig[1] and others, maintain that the egg is merely an interstitial cell which, after the ovary has begun to grow, increases in size more rapidly than its fellows. The egg seems in my sections always recognizable as such in the adult hydra. It is slightly larger than the inactive interstitials, has a very large nucleus in proportion to the cell body, adjacent to which there lies in this early stage a small dark ovoid body. The cell outline is spherical, whereas the resting interstitials are polygonal in section. There often appears at the early stage and always a little later, as Kleinenberg pointed out, a vacuole near the nucleus. All gradations from the large undoubted egg to this cell are readily found. But intermediates between it and the interstitials are, I may not yet say, wanting, but certainly rare. The evidence seems to point then to distinct germ cells in the adult hydra.

6. My results, furthermore, disagree with Kleinenberg's interpretation of the nutrition of the egg and extend the observations on the origin of the yolk granules as follows: At first the egg is nourished as are the adjacent ectoderm and interstitial cells. Material absorbed by the endoderm cells is massed in spherules filled with brown droplets, perhaps granules. This is apparently transformed, in large measure, in the endoderm cells and passed to the ectoderm, as this brown material seldom appears in the ectoderm cells. The endoderm cells elaborate also a material which stains deeply with osmic acid. This is also passed to the ectoderm, the cells of which are more heavily laden with it than are the endoderm cells. It passes into the egg and interstitial cells as well as others. The usual lecithin tests show it to be that substance or a closely related one. The egg at first contains the lecithin diffuse, but later in granular masses, "Pseudozellen." The interstitial cells also absorb it and the nuclei become filled with it, meantime enlarging considerably. Those interstitial cells adjacent to the egg in the fairly mature ovary have their walls in contact with the egg resorbed and the content of the cell becomes

[1] "Über Knospung und Geschlechtsentwicklung von *H. fusca*," *Biol. Centralbl.*, Vol. 26, 1906.

part of the egg (Nussbaum). The greatly enlarged nuclei, gorged with lecithin, also become yolk granules or "Pseudozellen."

7. Two polar bodies are extruded by the egg, as other observers have noted. The material thus far examined will not permit a final statement as to the details of reduction, but the first polar body contains twelve chromosomes, the second six. Certainly the female pronucleus has six, as has the male pronucleus also.

8. The nucleus and chromosomes though varying greatly in size are constantly present, never disintegrating and disappearing.

NOTES ON THE IDENTIFICATION OF THE CHÆTOGNATHA.

E. LE ROY MICHAEL.

Introductory Remarks.

It is the testimony of all, who have attempted the identification of the Chætognatha, that, for so small a group, they offer an immense amount of difficulty. The several species are very similar in appearance so that one is compelled to seek among details of structure for valid taxonomic characters. There is, moreover, considerable variation in most of the characters ; some of the valuable diagnostic features are readily destroyed even with the best possible preservation, and the methods of microscopical technique, usually so efficient, fail, for the most part, with the Chætognatha. It is not surprising then, that, without most careful examination, it becomes easy to base identifications on abnormalities or upon too variable characters. This has been repeatedly done by the earlier investigators with the result that the various species have become so entangled in the literature that, for anyone not a specialist on the group, identification is well nigh an impossible task. To meet this need for an adequate and ready means of identification the keys and tables herein presented have been prepared.

Except in the papers of Fowler ('05, '06), Hertwig ('80), and Krumbach ('03) identification has been based upon characters having little or no taxonomic value. Hertwig's investigations are clear and to the point but, unfortunately, only a few species came under his observations. Krumbach has accurately made a detailed study of the seizing jaws and has developed a classification based upon the minute anatomy of these structures. It is an exceedingly valuable contribution for, in many cases of poor preservation, these hard structures offer the only means of identification. There is, however, one difficulty with the Krumbach system : it requires so minute an examination that it is only after one has worked at it for some time that he is able to see the distinctive

features with certainty. Fowler's papers, accurate and careful in most details, offer, by far, the best aid to the systematist. The characters used are carefully chosen and tested by an examination of a large series of individuals of each species. Anyone attempting to work on this group will find both the Biscayan and Siboga Reports of this investigator invaluable.

In the genus *Sagitta* I have adopted the synonymy of Fowler ('06), who recognizes eighteen valid species, as shown in the table at the close of this paper. In addition there are several doubtful species: *Sagitta hispida* Conant, *S. tenuis* Conant, *S. maxima* Conant, *S. bedfordi* Doncaster, *S. septata* Doncaster, *S. elegans* Verrill, and *S. arctica* Aurivillus. Descriptions of these species have been so incomplete and drawings so few that, upon the available data, it is impossible to determine their validity. *S. arctica* is very possibly a synonym for *S. elegans* but, until the original specimens can be redescribed, it is best to leave them in the category of doubtful species. Of all the doubtful species Verrill's *S. elegans* appears to be the most valid, but so little is defined in the description that it will not be included in this paper.

Of the eighteen species only a few have, as yet, been found in American waters. *Sagitta hexaptera*, *S. enflata*, and the doubtful species *S. elegans*, *S. tenuis*, *S. maxima*, and *S. hispida* have been recorded from the Atlantic Coast, by Conant ('95, '96), Verrill ('83), and Stevens ('05). The genus *Spadella* is represented by two species, *Spadella draco*, and the doubtful *S. schizoptera* Conant. *Krohnia* is represented by the single species *Krohnia hamata*. The collections of the University of California from the San Diego region of the Pacific Coast, examined by me, contain the following species of *Sagitta*: *Sagitta serratodentata*, *S. zetesios*, *S. enflata*, *S. hexaptera*, *S. bipunctata*, *S. furcata*, *S. neglecta*, *S. decipiens*, and *S. pulchra*. *Spadella* is represented by the single species *Spadella draco*, only one individual having been taken. *Krohnia* has not, as yet, been recorded.

Most of the material, which I have examined, consists of a large number of specimens obtained from the coast of Southern California by the explorations of the San Diego Marine Biological Laboratory, and from the collections made by the "Albatross" in the explorations of the United States Bureau of Fisheries on

the coast of California in 1904. The method has been to separate the material into three assortments, according as the preservation was excellent, fair, or poor. A large series from each assortment was examined in an attempt to isolate the taxonomic characters, and the results from each individual specimen tabulated. Proceeding on this basis I am in a position to utilize, in all essentials, the characters Fowler ('05, '06) has chosen. In the ensuing pages frequent use will be made of his reports.

Methods of Preservation.

Killing has been tried with various combinations of acids, alcohol, and formalin, but, of all reagents, weak formalin gives, by far, the best results. Other reagents cause unequal contractions, swellings, and gross distortions, thereby ruining the material. Fowler ('05) advises killing separately in weak formalin then, after a short time, transferring them, for permanent preservation, into from 5 to 10 per cent. On the whole this method gives excellent results but I have found, in some cases, that considerable curling results. Specimens in this condition are difficult to handle and measurements are rendered more or less inaccurate. The following method will overcome this curling and otherwise give most perfect results: Each *Sagitta* is placed separately on a dry slide or cover-glass and allowed to remain exposed to the air for several seconds. This causes the animal to stick slightly to the slide. Then hold the slide in a vertical position and apply formalin (5 to 10 per cent.) at the upper end, allowing it to wash over the animal which will be killed before it has time to loosen itself from the slide. Another method of adding formalin, drop by drop, to a jar of *Sagitta* has given excellent results, but, if the formalin is added too fast, the results are uneven, owing to variability in the activity of the individuals, some contracting violently to a very weak solution while others are apparently unaffected by it. The three methods herein stated are all good; the second takes the most time but produces the surest results.

Method of Measurement.

Fowler ('05) advises camera drawings as measurements otherwise taken are frequently erroneous, in many cases not tallying

with the drawings at all. For the general features of the body the camera is necessary, but some measurements are so difficult to discern that one is never quite sure he is tracing correctly. I have found it extremely difficult, in such species as *Sagitta enflata*, to see the cephalic limits of the anterior fin, and have never been able to trace the outline with certainty. I find that an ocular micrometer gives more certain results, with such measurements, than the camera.

Characters Used in Classification.

The internal organs of *Sagitta* consist of the digestive, reproductive, and nervous systems. The first includes a simple straight tube leading from the mouth to the anus and offers no definable diagnostic characters; the two lateral diverticula, used by several writers, would seem to be not real diverticula, but rather results of extreme contraction of the head at death. The size and shape of the vesiculæ seminales depend entirely upon the sexual condition of the animal at the time of capture. The extension of the ovaries varies in the different species but, as they are the last organs to develop, one may find an otherwise mature *Sagitta* with ovaries of any length up to the specific maximum. The shape of the ovaries, whether long and slender, or short and thick, possibly has some significance, but as the length depends largely upon the extent of growth, and the width largely upon the maturity of the ova, too much weight cannot be placed upon these characters. The ova might possibly offer excellent characters for diagnosis, but with our present knowledge, one can never be sure whether they are mature or not. The nervous system, if well worked out for every species, might offer excellent characters, but except for two or three species the nervous system has not been investigated. Should the most excellent characters be offered here, their adoption as a means of ready identification would be inconvenient, owing to the technical methods and delicate work required to bring out the points. We are, therefore, compelled to look to the external characters as our only means of accurate identification.

Even among the external features a few characters have been used in the past, which appear to be worthless for specific deter-

mination. The comparative size of the head would seem to be due to the extent to which contraction occurs at death; if the contraction be slight the head appears larger, if great it appears smaller. This also applies to a less extent to the presence or not of a neck but, allowing for the variation thus produced, it may have some taxonomic significance. Again, the presence or absence of color seems to vary with the individual rather than with the species. Fowler ('05) has obtained, in the same haul, individuals of the same species, some a salmon pink, and others without color. The shape of the fins, whether triangular or half elliptical, would be an excellent character were the fins not so frequently damaged. Fowler ('05) has obtained specimens with a triangular fin on one side which had been rubbed into an ellipse on the other. One is often still less certain, from evidence derived from preserved material, whether the tail fin is truncate or rounded in nature.

The structural features then, which are available for diagnosis, consist of the cephalic armature, musculature, lateral fields, corona ciliata, and proportional measurements of various regions of the body. The table, at the close of this paper, includes all valid toxonomic characters, a discussion of which follows.

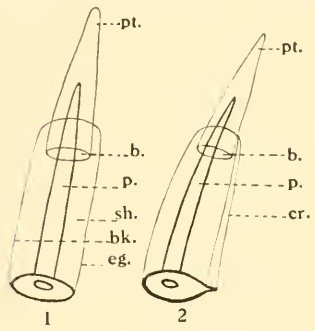
Prior to Krumbach's ('03) paper on "Die Greifhaken der Chaetognathen" practically the only use made, in classification, of the seizing jaws consisted of their enumeration and, even so, the tendency has been to describe the species on a basis of one or two individuals so that not enough latitude has been left for variation within the species. The number of seizing jaws is a very important matter and should be tabulated, together with the number of teeth and length of specimen, for a considerable number of individuals. When the number of seizing jaws is combined with their anatomical characters, as elaborated by Krumbach ('03), they present excellent criteria for identification, and, in many cases, where preservation is poor, practically the only safe criteria. Krumbach ('03) has defined the differences among the various species in the form and curvature of the seizing jaws, the presence and extent of a crest along the shaft, presence and nature of serrations, curvature and shape of the points, extent to which the point is inserted into the shaft, extent

of the pulp into the point, and the pattern of the pulp. Unfortunately all known species were not studied by Krumbach. His method should certainly be extended to the remaining species. His classification is here briefly summarized.

The nine species of Chætognatha studied by Krumbach ('03) were *Sagitta bipunctata*, *S. enflata*, *S. hexaptera*, *S. furcata*, *S. serratodentata*, *S. minima*, *Spadella draco*, *Krohnia hamata*, and *Sagitta magna*, the latter, according to Krumbach ('03), and Fowler ('06), probably being a variety of *S. hexaptera*. These several species Krumbach divides into four groups as follows:

GROUP 1 (Figs. 1 and 2).

Point *pt* with an oval base *b*: strongly needle-shaped. Pulp *p* extends along the central axis of the shaft. Upper third of shaft strongly bent. This group includes two species, *Sagitta bipunctata*, and *Spadella draco*.



Sagitta bipunctata (1).

Point *pt* imbedded one third its height into the shaft *sh*. Shaft with fine longitudinal furrows on the surfaces between the back *bk* and the edge *eg*.

Spadella draco (2).

Flat broad edged crest *cr* on edge of shaft. Point *pt* inserted one fourth to one fifth its height into shaft. Old jaws have serrations on the lower end of the shaft, which dwindle in size as they approach the point, disappearing entirely while still some distance from the point.

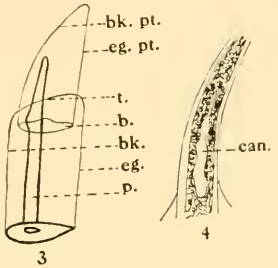
GROUP 2 (Figs. 3, 4 and 5).

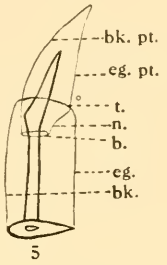
Point *pt* with an oval base *b*. Back of point *bk.pt* has greater curvature than the back of the shaft *bk*; edge of point *eg.pt* and edge of shaft *eg* have the same curvature so that the junction of the back and edge of the point at the apex lies toward the edge. Pulp *p* runs slightly nearer the back of the shaft *bk*. Shaft evenly

and slightly curved. This group includes *Sagitta enflata* and *S. furcata*.

Sagitta furcata (3 and 4).

Base of point *b* and top of shaft *t* converge as they approach the back of the shaft *bk*. Cross-section of the shaft is a slender wedge-shape. Pulp *p* is displaced toward the back of the shaft

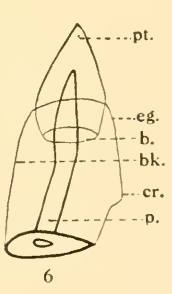


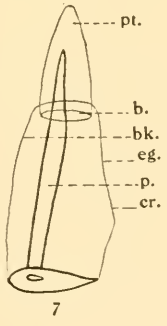


bk. Pulp *p* is concentrated around a canal *can*, which is of a cucumber form and extends to the upper third of the shaft. Old jaws with a small crest.

Sagitta enflata (5).

Base of point *b* and top of shaft *t* converge as they approach the edge of the shaft *eg*. Edge of point *eg.pt* near the base *b* is notched *n*. Cross-section of the shaft more of an oval than in *S. furcata*. Canal irregularly distributed through the pulp.





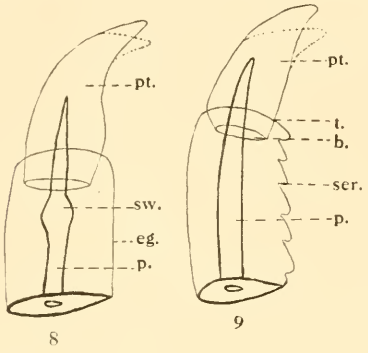
Group 3 (Figs. 6 and 7).

Point *pt* with a broad oval base *b*. Pulp *p* slightly toward back of the shaft *bk*. Base of point *b* makes a right angle with the back of the shaft *bk*. Angle between base of point *b* and edge of shaft *eg* is acute. Shaft below point with a short massive crest *cr*. Edge of crest extends proximally on a line with the edge of the point for some distance and then makes an abrupt turn toward the shaft. Shaft evenly and strongly

curved. This group includes *Sagitta hexaptera* (6) and *S. magna* (7). The latter is, as previously mentioned, now regarded as a variety of *S. hexaptera*. The only difference is that the jaws of *S. magna* are finer, more slender, and delicate than in an individual of *S. hexaptera* of the same size. The pulp reaches higher toward the apex of the point than in *S. hexaptera*.

GROUP 4 (Figs. 8, 9 and 10).

Point *pt* sickle-shaped, bent toward the edge of the shaft *eg*, varying in curvature between the two extremes indicated. Base more or less oval. Pulp *p* enters the shaft between the center



and the back focus of the cross-section. Pulp reaches nearly to the knee of the point, but never makes the bend. Shaft only slightly curved. This group includes *Sagitta minima*, *S. serratodentata* and *Krohnia hamata*.

Sagitta minima (8).

Jaws slender and long with slender points only slightly set into the shaft. Cross-section of shaft wedge-shaped. Pulp *p* is swollen *sw* slightly below the point. Tip of point *pt* bent much more than the rest of the jaw. Old jaws have a small delicate crest.

Sagitta serratodentata (9).

Pronounced serrations *ser* on the shaft. The first tooth of the serration is the smallest, extending from the top of the shaft *t* half-way to the basal line of the point *b*. Proceeding proximally the teeth increase in size. Cross-section of the shaft is an

elongated wedge. Old jaws are supplied with a pronounced crest.

Krohnia hamata (10).

Jaws heavy with broad oval cross-section. Pulp *p* only scantily fills the center of the canal *can*. Pulp swollen *sw* as in *S. minima*. Base of point *b* and top of shaft *t* converge toward the edge of the shaft *eg*.

Taken in connection with the seizing jaws the teeth afford an excellent means of identification. Their arrangement into two pairs of rows distinguishes *Spadella* and *Sagitta* from *Krohnia*, which has but one. Within the genus *Sagitta* a glance at the table at the close of this paper will indicate the importance of mere number. It is advisable that the limits of variation for every length of individual be accurately known. Were this accomplished, by combining the number of anterior and posterior teeth with the length of individual, a criterion of identification would result of much value to the systematist.

The form and arrangement of the teeth, if properly recorded, will considerably assist in identification. Some teeth are slender, some broad, so that proportional measurements of width and length are desirable; the length of tooth should also be compared to the length of individual so as to define exactly what is meant by saying that the posterior teeth of *Sagitta hexaptera* are long and slender, while those of *S. ferox* are long and broad. In any group like the Chætognatha, where, at best, identification is difficult, we should endeavor to rid ourselves of characters based upon mere comparisons. All such characters may, at the expense of a little labor, be reduced to exact or proportional measurements. If one merely says that the posterior teeth of *Sagitta siboga* "are long, broader, and with narrower bases than in *bedoti*," anyone attempting to identify a *S. siboga* would wonder whether the teeth were absolutely broader, broader in proportion to the length of tooth, or broader in proportion to the length of individual.

The inclination of the teeth, whether upright, externally or internally oblique; the proximity of setting, whether close or distant, are of some importance. Fowler ('05, '06) has used

some of these characters but, as yet, they are in a comparative form lacking in that element of exactness and definition so necessary for accurate classification by one unfamiliar with the group.

Another important character is present in the vestibular ridge. The presence of high or low, numerous or few, blunt or acute papillæ are matters that need more attention in the future. The position and height of the papilla should be compared to the position and height of the tooth, and similarly their number should be compared to the number of teeth. Comparison of the extent of the ridge with that of the tooth row proves of some importance; whether it is longer or shorter than the tooth row, and if shorter how many teeth project beyond; whether it terminates abruptly or gradually, in a lateral process or not.

The fins offer good characters if the specimen is well preserved. The presence or absence of a second pair of lateral fins serves to distinguish between *Sagitta* and the other two genera. Relation of the anterior to the posterior fins in length and breadth has been found to be a useful character. In *Sagitta hexaptera*, *S. macrocephala*, *S. neglecta*, *S. bipunctata*, *S. robusta*, *S. serratodentata*, and *S. planctonis* the posterior fin is longer then the anterior fin; in *Sagitta ferox*, *S. pulchra*, *S. zetesios*, *S. whartoni*, and *S. siboga* the posterior fin is shorter; in *Sagitta bedoti*, *S. enflata*, *S. regularis*, *S. furcata*, and *S. decipiens* both fins are the same length. Here again, it is necessary to know how much variation to allow for those in the third group, and to accurately determine this requires measurements of a large series of individuals of each species.

The distance from the anterior to the posterior fin, as measured in per cent. of the total length of the individual, may be made of considerable service. From the table we see that, in *Sagitta bedoti*, this distance measures but 5.4 per cent., the least I have found, except in the case of *Sagitta whartoni* where the fins are confluent, while *S. siboga* measures approximately 15 per cent. The other species are distributed between these extremes. While the measurements are not extensive enough to be of much value, still we can separate the species into two groups; those in which this interval is more than eight per cent. and those in which it is less than eight per cent. In making this separation we still

allow sufficient variation to feel quite safe, except in the case of *Sagitta bipunctata* (7.9 per cent.) and possibly *S. serratodentata* and *S. macrocephala* (7.5 per cent.).

The limits of the anterior fin as tested by the ventral ganglion, and the position of greatest width are of some value to the systematist, in individuals well preserved. In *Sagitta ferox*, *S. neglecta*, *S. pulchra*, *S. whartoni*, *S. serratodentata*, *S. siboga*, *S. zetesios*, and *S. planctonis* the fin extends to the ganglion; in *Sagitta enflata*, *S. hexaptera*, and *S. bipunctata* the fin is remote from the ganglion. The remaining species occupy various points between these two extremes. Until the extent of this variation is better known, for each species, separation cannot be rendered any more definite, by this criterion.

The posterior fin is more instructive. The position of greatest width, as tested by the tail septum; the proportion of posterior fin in front of the septum; the proximity of the fin to the vesiculæ seminales, are all serviceable characters. The table shows the grouping of the species in this matter.

The corona ciliata has been considerably used in the past. Length and width in proportion to the total length, shape and location, how much on head, how much on body, — these form the important features. The great difficulty in using this character lies in the fact that it is very rarely present in preserved material. With living material the corona might have considerable significance, but with formalin material nearly all specimens have apparently lost the structure.

The collarette and lateral fields are of some utility. The collarette or neck fin is an expansion of the ectoderm in the region of the neck and appears as a constant specific character. Some species are always provided with it as *Sagitta siboga*, *S. ferox*, *S. neglecta*, *S. regularis*, *S. pulchra*, *S. robusta*, *S. zetesios*, and *S. decipiens*. It is absent in the remaining species. The lateral fields are those areas between the muscles so that, in general, the presence of large lateral fields is co-existent with weak muscles. Species in this category are very often flabby and transparent in formalin. Formalin acts upon the muscles causing opacity and firmness so that the species with strong longitudinal muscles are readily separated from those with weak mus-

cles. The table indicates the distribution of these characters among the species.

Finally the general shape of the animal is of prime importance. Length, breadth, proportional length of tail, extent or absence of a constriction at the tail septum, are all points to be noted. In some species the body tapers gradually from head to tail, in others a sudden diminution occurs at the tail septum; some are of the same width throughout the entire trunk, others are much wider in the middle of the trunk tapering toward head and tail. Matters of this kind, while extremely useful, are difficult of diagnostic description so that camera drawings present the most accurate means of exhibiting these relations.

KEY TO THE GENERA OF CHÆTOGNATHA.

1. Two pairs of lateral fins. Two pairs of rows of teeth. Only slight epidermal thickening on body ..... *Sagitta.*
2. One pair of lateral fins, partly on body and tail. One pair of rows of teeth. Body longer than tail. No epidermal thickening behind the head..... *Krohnia.*
3. One pair of lateral fins, entirely upon the tail segment. Two pairs of rows of teeth. Prominent thickening of epidermis extending from behind head to tail. *Spadella.*

KEY FOR THE DETERMINATION OF THE SPECIES OF SAGITTA TO BE USED FOR LIVING OR PERFECTLY PRESERVED MATERIAL.

1. Species with collarette..... 12.
1. Species without collarette..... 2.
2. Shaft of seizing jaw serrated..... *Sagitta serratodentata.*
2. Shaft of seizing jaw not serrated..... 3.
3. Irregular transverse septa present on trunk..... *Sagitta minima.*
3. Irregular transverse septa absent on trunk..... 4.
4. Posterior teeth 12 to 32 in number..... 5.
4. Posterior teeth 1 to 12 in number..... 8.
5. At least 50 per cent. of the posterior fin in front of the septum..... 6.
5. Less than 50 per cent. of posterior fin in front of septum..... *Sagitta bedoti.*
6. Tail 28 to 40 per cent. of total length..... *Sagitta macrocephala*
6. Tail 16 to 25 per cent. of total length..... 7.
7. Middle third of body of equal width..... *Sagitta bipunctata.*
7. Body much wider at middle of the length..... *Sagitta enflata.*
8. Length of anterior fin from 44 to 66 per cent. of total length, confluent with the posterior fin. ..... *Sagitta whartoni.*
8. Length of anterior fin less than 40 per cent. of total length, always an interval between anterior and posterior fins..... 9.
9. Anterior fin on a level with ventral ganglion..... *Sagitta furcata.*
9. Anterior fin remote from ventral ganglion..... 10.
10. Posterior teeth less than 8 in number ..... *Sagitta hexaptera.*

10. Posterior teeth more than 8 in number ..... 11.
11. Middle third of body of equal width ..... *Sagitta bipunctata.*
11. Body much wider at middle of length..... *Sagitta enflata.*
12. Irregular transverse septa present on trunk ..... *Sagitta minima.*
12. Irregular transverse septa absent on trunk ..... 13.
13. At least 50 per cent. of posterior fin in front of septum..... 14.
13. Less than 50 per cent. of posterior fin in front of septum..... 18.
14. Posterior fin widest in front of septum..... *Sagitta zetesios.*
14. Posterior fin widest at or behind septum..... 15.
15. Posterior fin shorter than anterior fin..... ..... 16.
15. Posterior fin as long or longer than anterior fin..... 17.
16. Posterior teeth 9 to 15 in number .... *Sagitta pulchra.*
16. Posterior teeth 15 to 23 in number ..... *Sagitta siboga.*
17. Anterior fin reaching to middle of ventral ganglion ..... *Sagitta planctonis.*
17. Anterior fin not reaching to ventral ganglion..... *Sagitta decipiens.*
18. Posterior fin shorter than anterior fin..... ..... *Sagitta ferox.*
18. Posterior fin as long or longer than anterior fin..... 19.
19. Corona ciliata extends anteriorly beyond eyes .... *Sagitta robusta.*
19. Corona ciliata not extending to eyes..... 20.
20. Middle third of body of equal width..... *Sagitta neglecta.*
20. Body of equal width from the ventral ganglion to the seminal vesicles. *Sagitta regularis.*

If (19) the corona ciliata is absent the following criteria may be used :

19. Middle third of body equally wide..... *Sagitta neglecta.*
19. Body equally wide from ventral ganglion to the seminal vesicles..*Sagitta regularis.*
19. Body equally wide from just behind the neck to immediately in front of the tail septum..... *Sagitta robusta.*

KEY FOR DETERMINATION OF THE SPECIES OF SAGITTA TO BE USED FOR POORLY PRESERVED MATERIAL.

1. Species with collarette..... ..... 12.
1. Species without collarette..... 2.
2. Shaft of seizing jaws serrated..... *Sagitta serratodentata.*
2. Shaft of seizing jaws not serrated..... ..... 3.
3. Irregular transverse septa present on trunk..... *Sagitta minima.*
3. Irregular transverse septa absent..... 4.
4. Length of anterior fin from 44 to 66 per cent. of the total length. anterior fin and posterior fins confluent..... *Sagitta whartoni.*
4. Length of anterior fins never as much as 40 per cent. of total length, always an interval between anterior and posterior fin..... 5.
5. Tail 16 to 27 per cent. of the total length..... 6.
5. Tail 28 to 35 per cent. of total length..... 7.
6. Posterior teeth less than 8 in number..... 8.
6. Posterior teeth more than 8 in number..... 9.
7. Jaws more than 8 in number..... *Sagitta macrocephala.*
7. Jaws less than 8 in number..... *Sagitta bedoti.*
8. Anterior teeth 1 to 4 in number..... *Sagitta hexaptera.*

8. Anterior teeth more than 4 in number.....*Sagitta furcata.*
9. Body firm and opaque; tail slender.....*Sagitta bipunctata.*
9. Body transparent in formalin.....10.
10. Body flabby and easily compressed.....*Sagitta furcata.*
10. Body firm.....11.
11. Neck very marked.....*Sagitta enflata.*
11. Neck almost missing.....*Sagitta bedoti.*
12. Irregular transverse septa present on trunk.....*Sagitta minima.*
12. Irregular transverse septa absent.....13.
13. Neck well marked.....14.
13. Without neck.....18.
14. Jaws more than 8 in number.....15.
14. Jaws less than 8 in number.....16.
15. Collarette extends to anterior fin.....*Sagitta zetesios.*
15. Collarette never extends over half the distance to the anterior fins. *Sagitta planctonis.*
16. Body thickest in the middle third.....*Sagitta siboga.*
16. Body of uniform thickness from ventral ganglion to tail.....17.
17. Collarette extends to anterior fins.....*Sagitta ferox.*
17. Collarette never extends over half the distance to the anterior fins. *Sagitta robusta.*
18. Posterior teeth less than 7 in number.....*Sagitta regularis.*
18. Posterior teeth more than 7 in number.....19.
19. Body opaque.....20.
19. Body transparent.....21.
20. Body equally thick from just behind neck to the tail septum.....*Sagitta robusta.*
20. Body thicker in the middle third, gradually tapering toward head and tail. *Sagitta neglecta.*
21. Body width about 10 per cent. of total length, tail less than 27 per cent. of total length.....*Sagitta pulchra.*
21. Body width about 5 per cent. of total length, tail more than 27 per cent. of total length.....*Sagitta decipiens.*

Key for the Determination of the Species of Krohnia.

1. Lateral fin extends from the region of the ventral ganglion to somewhat behind the tail septum; never reaches to seminal vesicles; more than 60 per cent. in front of tail septum.....*Krohnia hamata.*
1. Cephalic limit of lateral fin never more than half the distance from the tail septum to the ventral ganglion; extends caudally to the seminal vesicles; more than 40 per cant. of fin behind septum.....2.
2. Body width about 4 per cent. of total length. Tail 30 to 40 per cent. of total length. Seizing jaws evenly curved, 6 to 9 in number. Teeth 9 to 13. *Krohnia subtilis.*
2. Body width about 5 per cent. of total length. Tail 25 to 41 per cent. of total length (usually not over 33 per cent.). Teeth 11 to 15. Seizing jaws 6 to 9, with the convex edge made up of two curves, the junction of which makes an obtuse angle at a point about one fourth the length of the jaw proximad of the tip. *Krohnia pacifica.*

KEY FOR THE DETERMINATION OF THE SPECIES OF SPADELLA.

1. Average width of collarette nearly half that of the body, widest slightly anterior to the tail septum. Length of fin always less than 5 times its width. Anterior teeth 7 to 10. Posterior teeth 11 to 16.....*Spadella draco.*
2. Average width of collarette much less than half that of the body; widest slightly posterior to the head. Length of fin always more than 5 times its width. An terior teeth 3 to 5. Posterior teeth 3 to 4.....*Spadella cephaloptera.*

DESCRIPTION OF TABLE.

The data for this table have been gathered from various sources, but mostly from the investigations of Fowler ('05, '06) and Krumbach ('03). In proportional measurements, number of seizing jaws, and number of teeth, the extremes of variation have been indicated wherever possible.

In the case of *Sagitta whartoni* the data have been obtained from Fowler's "Contributions to our Knowledge of the Plankton of the Faroe Channel," 1896. In this instance a considerable discrepancy, due in all probability to a typographical error, is found in comparing the drawing with the descriptive measurements. From Fowler's description the width of the anterior fin varies from 10 to 15 per cent. of the total length; from his drawing it measures but 2.8 per cent. of the total length. Similarly, in the width of the posterior fin, his measurements show variation from 13 to 21 per cent. of the total length, while his drawing shows it but 5.7 per cent. In no other species has the width of the anterior fin been over 6 per cent. of the total length, nor the width of the posterior fin over 7 per cent. For this reason I have utilized the fin measurements as taken from the drawing rather than from the description of *Sagitta whartoni.*

I desire to express my obligations to Dr. C. A. Kofoid, through whose direction, advice, and criticism I have received invaluable aid.

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Explanation of Plate.

Fig. 1. *Sagitta hexaptera* × 2. *ant.t.*, anterior teeth; *post.t.*, posterior teeth; *s.j.*, seizing jaws; *al.can.*, alimentary canal; *vent.gang.*, ventral ganglion; *ant.f.*, anterior fin; *post.f.*, posterior fin; *t.s.*, tail septum; *t.*, tail; *t.f.*, tail fin.

Fig. 2. *Sagitta neglecta* × 2. *col.*, collarette; *vent.gang.*, ventral ganglion; *ant.f.*, anterior fin; *post.f.*, posterior fin; *t.s.*, tail septum; *t.*, tail; *sem.ves.*, seminal vesicles; *t.f.*, tail fin.

Fig. 3. *Sagitta robusta* × 13. (After Fowler.) *e.*, eye; *cor.cil.*, corona ciliata; *col.*, collarette; *vent.gang.*, ventral ganglion.

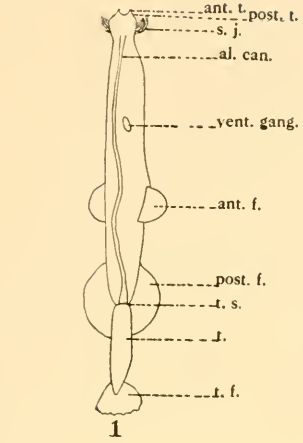
Fig. 4. *Sagitta regularis* × 22. (After Fowler.) *e.*, eye; *cor.cil.*, corona ciliata; *col.*, collarette; *vent.gang.*, ventral ganglion.

Fig. 5. × 346. Vestibular ridge and posterior teeth of *Sagitta neglecta*.

Fig. 6. Typical seizing jaw. (After Krumbach.) *pt.*, point; *ap.p.*, apical pulp; *out.mem.*, outer membrane; *cr.*, crest; *bk.*, back of shaft; *eg.*, edge of shaft; *p.*, pulp; *p.can.*, pulp canal; *vent.col.*, ventral column; *dor.col.*, dorsal column; *mus.at.*, muscle attachment; *b.p.*, basal pulp.

Fig. 7. *Spadella draco* × 8. *col.*, collarette; *vent.gang.*, ventral ganglion; *t.s.*, tail septum; *lat.f.*, lateral fin; *t.*, tail; *t.f.*, tail fin.

Fig. 8. *Krohnia hamata* × 3. (After Fowler.) *vent.gang.*, ventral ganglion; *lat.f.*, lateral fin; *t.s.*, tail septum; *t.*, tail; *t.f.*, tail fin.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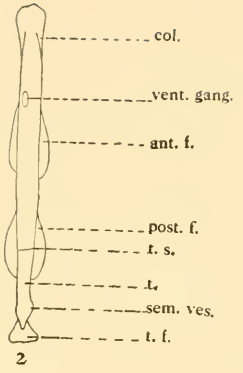
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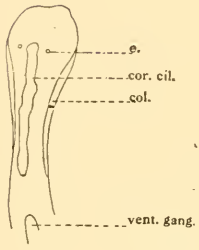
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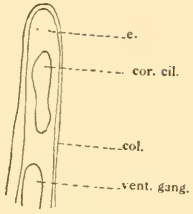
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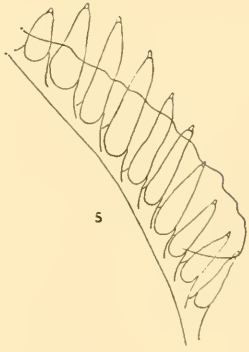

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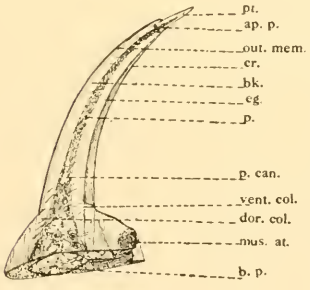
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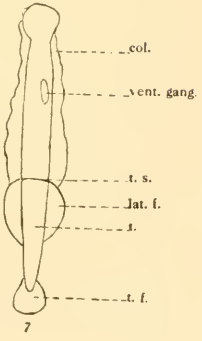
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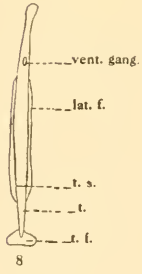
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8

EFFECTS OF ALCOHOL ON THE LIFE CYCLE OF INFUSORIA.

LORANDE LOSS WOODRUFF.

So many investigations have been undertaken to determine the effects of alcohol on the higher animals and with such varied results, that it is of interest to determine its effect upon the lowest and most generalized animal organisms, the Protozoa. As single-celled animals these forms cannot fail to give results of importance from the point of view of general cell-physiology, and lead to a clearer analysis of the primary effects of alcohol on metabolism. The Protozoa are particularly well fitted for cellular-physiological study, not only because as single free-living cells they lend themselves readily to experimental methods, but also because no one function predominates at the expense of the rest, and results obtained may reasonably be supposed to be due to the effects of the stimulus in question on the general metabolism of the cell.

Hunt[1] recently made some interesting experiments on the effect of small doses of alcohol on mice and guinea-pigs, and found that animals to which alcohol has been administered for some time acquire increased susceptibility to a definite poison (acetonitrile), and reached the conclusion that this increase in susceptibility is not due to a general "lowering of resistance," but is associated with a distinctly increased power of the body to break up the molecule of acetonitrile.

Calkins and Lieb[2] carried on some experiments with alcohol on *Paramecium* and found that ". . . alcohol has no effect when taken in too weak doses, and too powerful an effect when taken in over strong doses." ". . . when a medium dose is given (for example 3 parts of 1/1,000 alcohol to 2 of hay, or 1 part of

[1] Reid Hunt, "Studies on Experimental Alcoholism," Bull. No. 33, Hyg. Lab., U. S. Pub. Health and Mar.-Hosp. Serv., Washington, 1907.

[2] Gary N. Calkins and C. C. Lieb, "Studies on the Life-History of Protozoa,—II. The Effects of Stimuli on the Life-Cycle of *Paramecium caudatum*," *Archiv fur Protistenkunde*, 1902.

1/500 alcohol to 4 parts of hay) the effect is a continued stimulus which sustains the high rate of division even during periods of depression of the control series." "There is no doubt that for a time at least, alcohol will prevent death during periods of depression. . . ." ". . . there is evidence that . . . the general vitality would decrease under the constant stimulus as it does under treatment with hay infusion alone, although much more slowly." "Notwithstanding the more rapid living, the general vitality does not seem to be affected badly by the alcohol." It was chiefly to determine the latter point that this investigation was begun, and I shall outline the progress of the work to the present time.

II. Methods.

I chose *Paramecium aurelia*[1] for the main line of experiments chiefly because considerable work has already been performed on this organism; and because it is one of the more generalized of the ciliates; and lastly because its cosmopolitan distribution renders it a convenient form to be studied in all laboratories. It seems to me to be more desirable, in the present state of our knowledge, to learn one form thoroughly, if that is possible, rather than to distribute our energies over a broader field. As a subsidiary line, for comparison and as a check on the *Paramecium* cultures, I employed a culture of *Stylonychia mytilus*.

The general method of carrying the cultures is identical with that which has been described in detail in an earlier paper,[2] so that a brief outline at this time will suffice.

A "wild" individual was captured and placed on a depression slide in five drops of hay infusion. This infusion was made by putting about three grams of hay or grass in 200 c.c. of tap water and then raising the temperature to the boiling point. This infusion was generally used as soon as it had again attained the room temperature. It was made fresh daily as a rule. Sufficient bacteria developed to provide ample nourishment for the infusoria, and since all precautions were taken in selection of the hay, etc.,

[1] The specific name *aurelia*, instead of *caudatum*, is adopted in accordance with the data advanced by Calkins, "*Paramecium aurelia* and *Paramecium caudatum*," Biol. Stud. Pupils of W. T. Sedgwick, Chicago, 1906.

[2] Lorande Loss Woodruff, "An Experimental Study on the Life-History of Hypotrichous Infusoria," *Journal of Experimental Zoölogy*, II., 4, 1905.

it is believed that a satisfactory culture medium for comparative work was obtained. When the isolated protozoön had divided twice, producing four individuals, each was isolated on a separate depression slide and thus were started the four lines of which each culture consisted. Thus, for example, *Paramecium*, culture I, comprised four lines, *I–a*, *I–b*, *I–c* and *I–d*. These lines were thenceforth kept distinct unless one became extinct through the isolation of a weak individual or through accident, in which case the line was started again from one of the three surviving sister lines of the same culture.

The rate of division was recorded daily for each of the four lines, and at the time of record an individual from each line was isolated on a clean depression slide in five drops of hay infusion. In computing the rate of division of the culture as a whole, with which we are alone concerned, the four lines (*a*, *b*, *c*, *d*) of the culture were averaged together and this result was again averaged for five-day periods. By this method it is believed that a just conception of the rate of division of the culture was obtained, as the average of the four lines largely obliterated the fluctuations in the division rate of any one line, which may not have been of much significance, or which may have been merely due to the isolation of a weak individual. One who has carried on cultures of protozoa for considerable periods cannot fail to recognize the fact that, as is to be expected, individuals vary greatly in their general vitality, etc., and it is necessary to isolate representative individuals. It is here that the personal equation of the experimenter comes into view, and therefore it is desirable that the same person should make the daily isolations.

The culture slides were arranged in small moist chambers to prevent evaporation. As in previous experiments of this nature, the minimum and maximum temperature of the room in the vicinity of the cultures was recorded daily, as indicated by a registering thermometer. By averaging the minimum and maximum points of each day for five-day periods the results obtained are quite satisfactory for comparative work. In the experiments under consideration, as in those of previous investigators in this field, the rate of division was taken as the indication of the physiological condition of the organisms; it being generally accepted

that this is the most accurate indication of the general metabolic condition of the protoplasm of organisms which is available.

The work was started at the Thompson Biological Laboratory of Williams College in the spring of 1907; was continued at the Woods Holl Marine Biological Laboratory during the summer, and is at present being carried on at the Sheffield Biological Laboratory of Yale University.

III. Details of Control Cultures.

(*a*) *Paramecium aurelia.*

The "wild" individual with which this culture (*Paramecium* I) was started was isolated from an infusion in the Williams laboratory on May 1, 1907, and has been kept continually under observation since that time, and is at present (January 25, 1908) in the 332d generation. The average rate of division of the four lines (*I–a*, *I–b*, *I–c*, *I–d*), again averaged for each five-day period during the life of the culture up to present time, is plotted by the familiar block method. By glancing at Figs. 1 and 2 (continuous line) it will be seen that the culture started off with a division rate of just two divisions per day, and at the present time, period 54, is averaging one and three quarters divisions per day.

I shall not attempt to analyze the division rate of the culture in relation to the life cycle of *Paramecium* at the present time, as in the experiments under consideration it is of interest solely as the "control."

(*b*) *Stylonychia mytilus.*

This culture (*Stylonychia* I) was started with a "wild" individual found in an infusion in the Yale laboratory on October 23, 1907, since which time it has been under daily observation and has been subjected to the same method and treatment as the *Paramecium* culture. It is at present (January 25, 1908) in the 165th generation. The rate of division is plotted in Fig. 3.

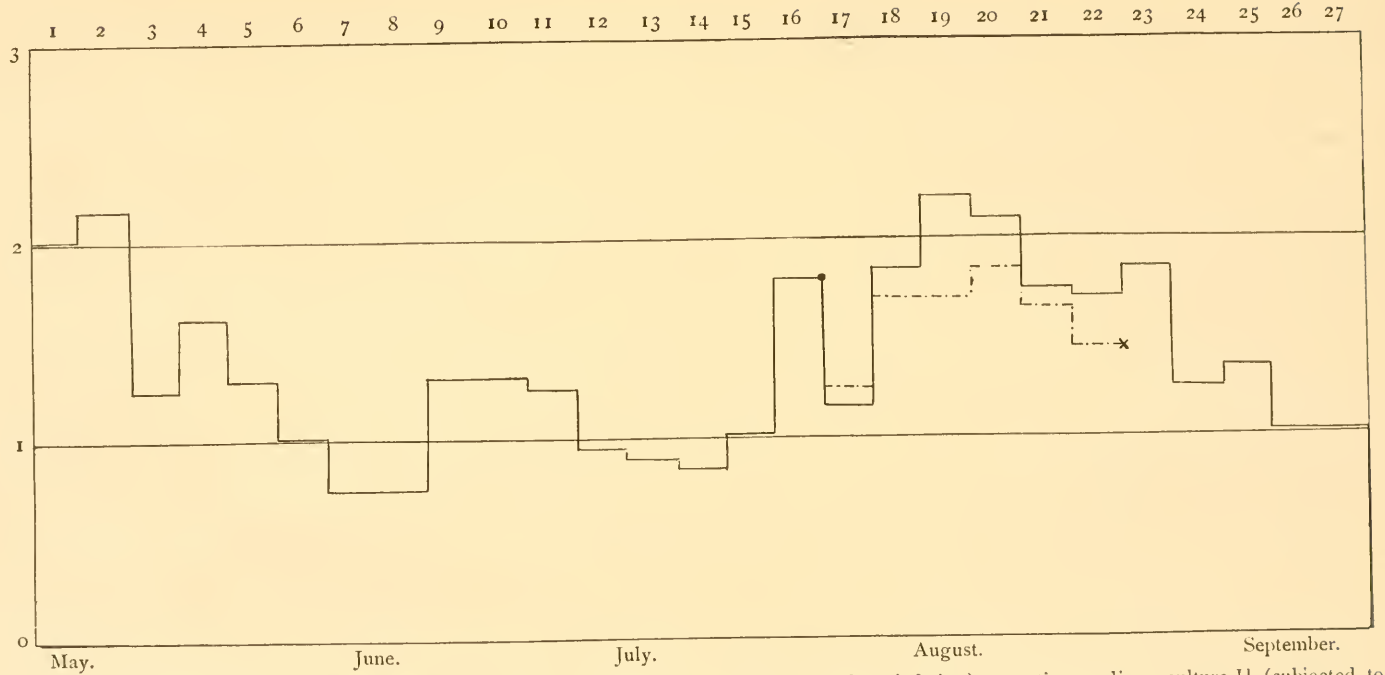

FIG. 1. Life-history of *Paramecium aurelia* (periods 1 through 27). Culture I (hay infusion) = continuous line; culture I^1 (subjected to 1/2,500 alcohol) = · · · · · line. ● = point of isolation of culture I^1 from culture I. × = point at which culture I^1 was discontinued. The figures above the diagram represent the five-day periods for which the rate of division is averaged. The figures at the left represent the average number of divisions per day of the four lines.

IV. General Effect of Alcohol on the Division Rate of Paramecium and Stylonychia.

(*a*) *Paramecium.*

On July 19, 1907, a second culture of *Paramecium* was started by isolation of an individual from each line of culture I. This second culture, designated *Paramecium* I^1, was carried under identically the same conditions as culture I, being subjected to the same temperature changes, etc., and given the same culture medium at the same time, except that instead of each individual receiving five drops of hay infusion (as in the case of culture I, the control), each received four drops of hay infusion and one drop of 1/500 alcohol. There were, then, two cultures each consisting of four lines which had been under observation for two and one half months. The only apparent difference in the conditions of the two cultures was that one was subjected to one part of alcohol to 2,500 parts of culture medium.[1]

The effect of the alcohol was seen in a slightly increased rate of division of culture I^1 (see Fig. 1, ·····line) above that of the control during the first five-day period. But after that, during the remaining twenty-five days of the experiment, the alcohol caused a decrease in the division rate. This culture was discontinued on August 19, 1907. In Table I is given the actual daily record of generations of the control culture and of the alcoholized culture, as an illustration of the general method of keeping the records of all the cultures throughout the work.

On November 6, 1907, another culture (I^2) was started by isolation from culture I and given the same treatment as that to which culture I^1 was subjected. A glance at Fig. 2 (·---·line) shows, however, that the effect of alcohol was this time a stimulation of the rate of division, for during the first thirty days of the experiment the rate was considerably more rapid than that of the control.

There is then, comparing the results of culture I^1 and those of the first thirty days of culture I^2, a clear-cut example of a stimulus (alcohol) causing a general retardation of the division

[1] Various amounts of alcohol were tried and the strength here employed was chosen as the one giving the best result. All strengths of alcohol which were tried at this period produced the same general effect.

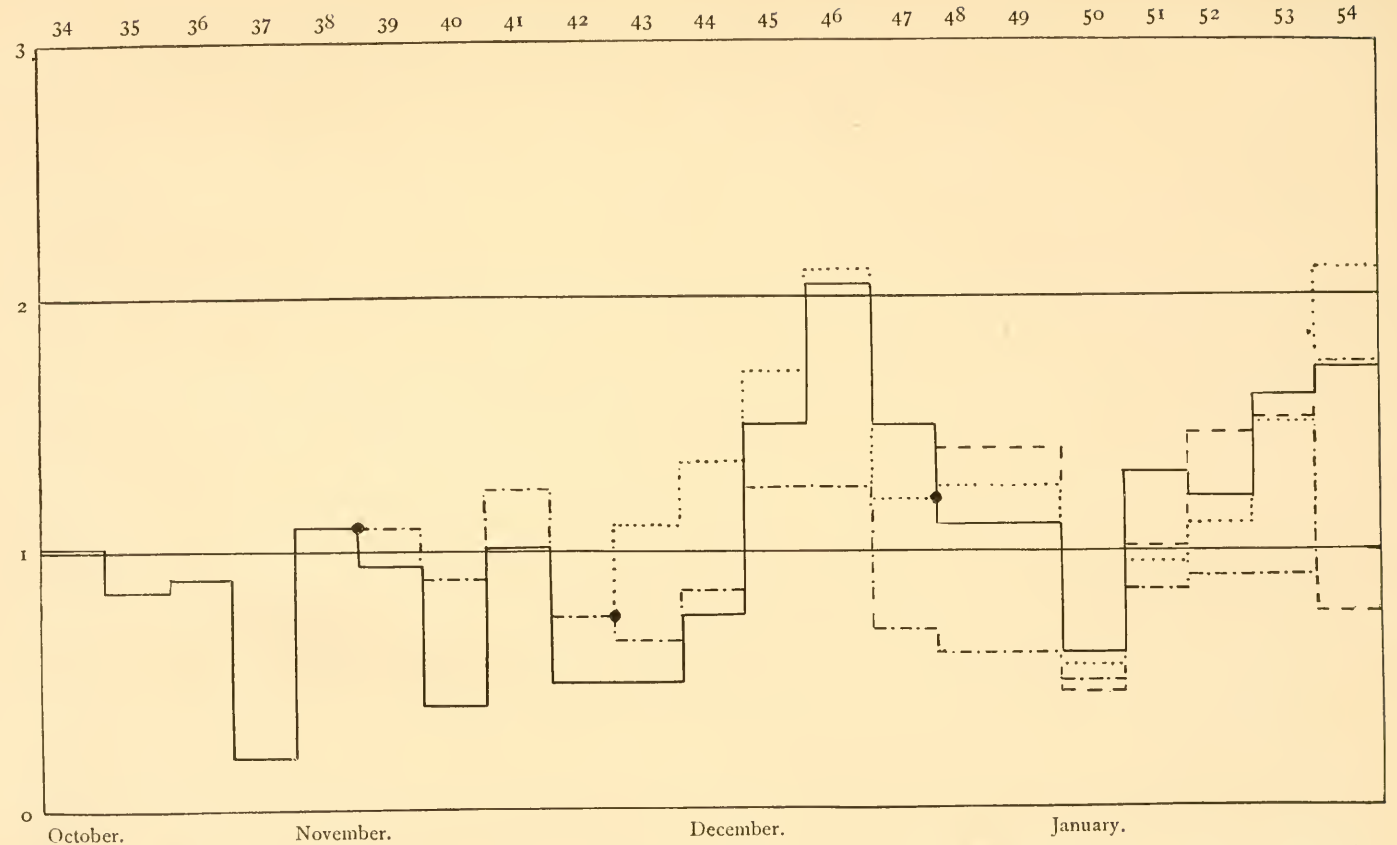

FIG. 2. Life-history of *Paramecium aurelia* (periods 34 through 54). Culture I (hay infusion) = continuous line; culture I^{2} subjected to 1/2,500 alcohol = · - · - · - line; culture I^{2a} subjected to 2/2,500 alcohol = · · · · line; culture $I^{2a'}$ subjected to 4/2,500 alcohol = - - - - - line. ● = points of isolation of the respective alcoholized cultures. Other details as in Fig. 1.

rate at one part of the life cycle and a general acceleration of the rate at another part.

During period 45, however, the rate of division of culture I^2

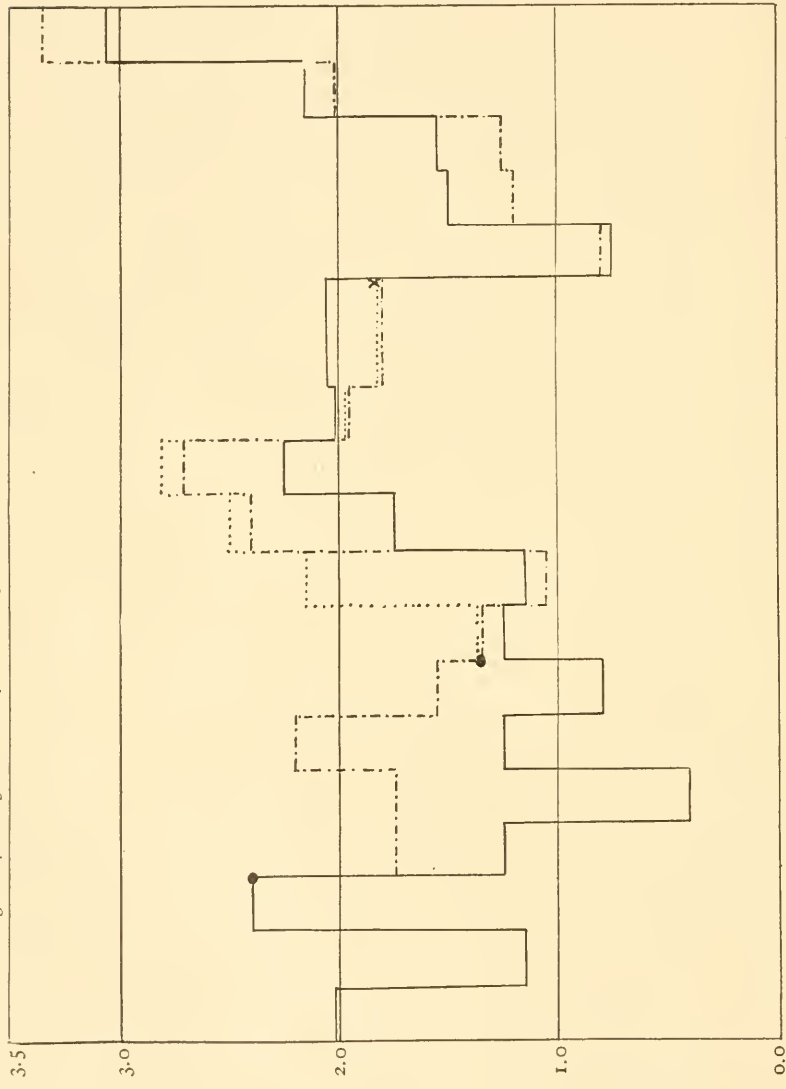

FIG. 3. Life-history of *Stylonychia mytilus* (periods 1 through 19). Culture I (hay infusion, control) = continuous line; culture I^1 subjected to 1/2,500 alcohol = · · · · · line; culture I^{1a} subjected to 2/2,500 alcohol = · · · · line; × = point at which culture $I^{1a'}$ was discontinued. Other details as in Fig. 1.

fell below that of the control (I) and has remained so up to the present period when it increased to the same rate as that of the control (Fig. 2, periods 45 through 54). It was suspected, how-

PARAMECIUM I.

Date.	July.													August.																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature Degrees F.	71/78	71/79	70/77	72/80	71/78	67/79	66/73	72/75	66/76	68/74	69/77	69/76	70/77	69/77	72/79	73/78	66/78	65/68	69/72	69/74	70/78	69/75	66/77	71/76	71/76	71/77	67/76	65/75	66/72	66/71	70/75
Line *a* generations	100	101	101	103	103	b 106	107	110	111	113	115	117	120	121	124	126	128	130	132	134	137	138	141	143	145	147	148	150	151	153	155
" *b* "	99	100	101	102	104	106	107	110	111	113	115	118	120	122	124	126	129	130	133	135	138	139	142	144	146	148	149	151	152	154	156
" *c* "	97	98	98	100	100	102	104	107	108	110	112	115	117	119	121	123	126	127	127	a 134	138	139	142	144	146	147	149	152	153	155	156
" *d* "	100	101	101	103	105	107	108	110	111	114	116	119	121	123	125	127	129	131	133	135	138	138	140	142	144	146	147	150	151	153	155

PARAMECIUM I[1].

Date.	July.													August.																	
	19	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature Degrees F.	71/78	71/79	70/77	72/80	71/78	67/79	66/73	72/75	66/76	68/74	69/77	69/76	70/77	69/77	72/79	73/78	66/78	65/68	69/72	69/74	70/78	69/75	66/77	71/76	71/76	71/77	67/76	65/75	66/72	66/71	70/75
Line *a* from *I–a*.	100	101	101	104	104	106	108	110	111	113	115	117	119	120	122	123	125	127	129	131	133	134	135	138	139	141	143	146	147	149	151
" *b* " *I–b*.	99	100	101	103	104	105	107	107	108	110	112	113	115	116	118	120	122	123	125	127	130	131	133	135	137	139	140	143	144	146	147
" *c* " *I–c*.	97	98	99	100	101	103	105	107	108	111	113	115	117	118	120	122	124	124	125	127	130	131	134	136	138	140	141	144	145	147	149
" *d* " *I–d*.	100	101	102	104	105	107	108	110	111	113	115	117	119	120	122	124	126	127	129	130	133	133	135	137	139	139	140	140	140	141	141

TABLE I.

Daily Record of Paramecium Cultures I and I[1] from July 19, 1907, to August 18, 1907.

ever, that the accelerating effect of a given strength of alcohol would not be continuous, so at period 43 another culture was started line by line from culture I^2 in identically the same way except that the amount of alcohol administered was doubled, each individual receiving one drop of 2/500 alcohol and four drops of hay infusion. The rate of division of this series, designated culture I^{2a}, at once greatly increased (see Fig. 2, · · · · line) and kept considerably above the control during the first twenty days of the experiment. During the succeeding periods it fluctuated above and below the control and at the present time is dividing considerably faster than the control culture (Fig. 2 · · · · line).

During the early part of culture I^{2a}, there was again reason to believe that the increase in the division rate would not be permanent, so still another culture ($I^{2a'}$) was isolated from culture I^{2a} (during period 48), and was treated with double the amount of alcohol to which the parent culture was subjected, that is, with one drop of 4/500 alcohol and four drops of hay infusion. The result was still again an initial increase in the rate of division, though of shorter duration, followed by fluctuations above and below the control series (Fig. 2, - - - - line).

In view of the fact that culture I^1 showed a practically uniform depression of the division rate when subjected to alcohol, it was thought that possibly the depression effect which secondarily appeared in cultures I^2, I^{2a}, and $I^{2a'}$, might be due to the fact that the culture as a whole again had attained a period in the life cycle when alcohol had a depressing effect on cell division and, therefore, that the falling off of the rate of division of the organisms, after being a certain length of time subjected to the alcohol, was not due to the animals becoming accustomed to the alcohol. To test this point another culture (I^3) was isolated from the control (I) and carried for three periods of five days each on one drop of 1/500 alcohol and four drops of hay infusion. During this time the division rate of culture I^3 was consistently accelerated and as greatly as that of culture $I^{2a'}$ which was receiving four times as much alcohol, thus showing that the results obtained in the alcohol experiments were due to "acclimatization," and not to the fact that the organisms were in a period of the cycle characterized by a changed susceptibility to alcohol.

(*b*) *Stylonychia.*

On November 6, 1907, a second culture of *Stylonychia* (culture I^1) was started line by line from culture I in exactly the same manner as *Paramecium* I^1 was started from *Paramecium* I. The treatment which followed was identically the same as that already described for the *Paramecium* experiments — culture I^1 being subjected to one part of alcohol to 2,500 parts of culture medium. The effect of this treatment is shown in Fig. 3, beginning at period 4. It will be noted that the division rate of the alcohol treated series (· - · - · line) was very much more rapid than that of the control (continuous line) during the first five five-day periods of the experiment. It fell slightly below the control during the next period, but during the following two periods of the experiment it was again far more rapid than the control. Thus we find in the case of *Stylonychia* that the treatment with alcohol of the strength employed produced, as in the *Paramecium* (culture I^2) experiment, stimulation for about the first month of the work. The plotted curve shows also that from this point on the rate of division of this series fluctuated above and below the control and at the present period it is again exceeding that of the control culture.

On November 26, 1907, culture I^{1a} was started from culture I, and was thenceforth treated with double the amount of alcohol (one drop of 2/500 alcohol and four drops of hay infusion) to which the parent culture was subjected. The result, again similar to that of the corresponding *Paramecium* culture, shows an increased division rate for several five-day periods of the culture subjected to the increased amount of alcohol. (Cf. Fig. 3, period 8 through 14, ····· line.) Again the stimulating effect was not continuous, but instead, as in the previously described experiments, the division rate of the alcohol-treated line finally fell below that of the control and remained below until the experiment was discontinued on December 31, 1907 ; though a new culture isolated from this culture and stimulated with double the amount of alcohol showed an increased division rate at first, and later decreased division rate as compared with that of the control culture.

V. Does Alcohol Cause a General Lowering of Resistance to Changes in the Environment?

Experiments to determine if treatment with alcohol will cause a general "lowering of resistance" to inimical changes in the environment are in progress, and the experiments described in this paper outline the method which is being employed in such a study.

For this work certain of the previously described cultures are being used, viz.,

Paramecium.	*Stylonychia.*
I = hay infusion.	I = hay infusion.
I^2 = hay infusion + 1/2,500 alcohol.	I^1 = hay infusion + 1/2,500 alcohol.
I^{2a} = hay infusion + 2/2,500 alcohol.	I^2 = hay infusion + 2/2,500 alcohol.
$I^{2a'}$ = hay infusion + 4/2,500 alcohol.	

On December 21, 1907, an individual was isolated from each line of the first three *Paramecium* cultures and from each line of the three *Stylonychia* cultures, and treated in identically the same way as the culture from which they were taken except that each was subjected to one part of copper sulphate in 1,250,000 parts of culture medium.[1] For example, the culture I^2_c isolated from *Paramecium* I^2 received daily three drops of hay infusion plus one drop of 1/500 alcohol plus one drop of 1/250,000 copper sulphate, *i. e.*, it received one drop of 1/250,000 copper sulphate in place of one of the drops of hay infusion received by culture I^2.

The six cultures isolated were then as follows:

Paramecium.	*Stylonychia.*
I_c = hay infusion + 1/1,250,000 $CuSO_4$.	I_c = hay infusion + 1/1,250,000 $CuSO_4$.
I^2_c = hay infusion + 1/2,500 alcohol + 1/1,250,000 $CuSO_4$.	I^1_c = hay infusion + 1/2,500 alcohol + 1/1,250,000 $CuSO_4$.
I^{2a}_c = hay infusion + 2/2,500 alcohol + 1/1,250,000 $CuSO_4$.	I^2_c = hay infusion + 2/2,500 alcohol + 1/1,250,000 $CuSO_4$.

The results of these experiments are plotted in Figs. 4–7. These curves show that, in the experiments on *Paramecium*, the alcohol-treated cultures (Figs. 5, 6) died out under the administration of copper sulphate during the fifth period of experimentation, that

[1] Various solutions of copper sulphate were tried and the one employed was selected because it appeared to be the maximum strength which all the cultures could withstand.

is, after being subjected to copper sulphate for twenty-three days, whereas the culture which had never been subjected to alcohol survived under the copper sulphate treatment until discontinued (Fig. 4). A closer analysis of the curves shows that during the earlier periods of the experiment the division rate of the organisms treated with 1/2,500 alcohol was somewhat less affected by the copper sulphate treatment than the division rate of the non-

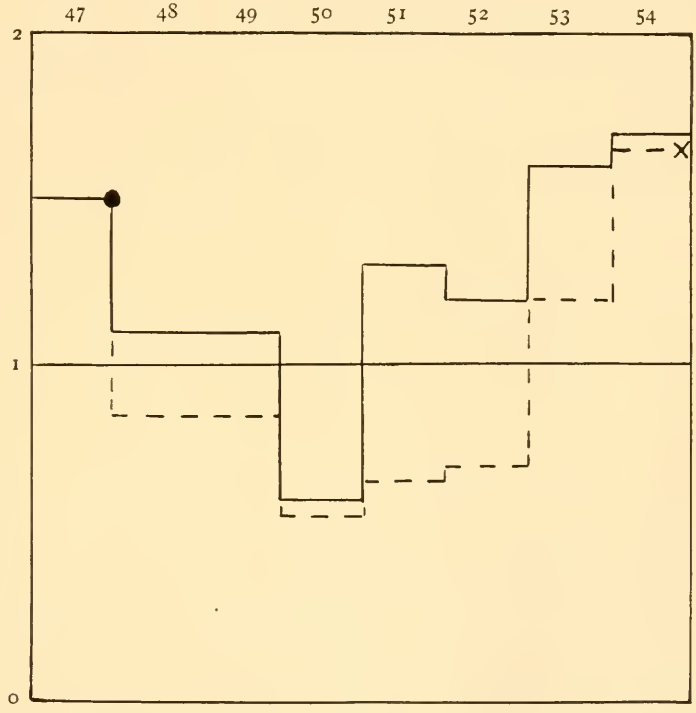

FIG. 4. *Paramecium.* Culture I (hay infusion, control) periods 47 through 54 = continuous line; culture I_c subjected to $CuSO_4$ = - - - - - line. × = point at which culture I_c was discontinued. Other details as in Fig. 1.

alcoholized line, but this was merely temporary. The lines subjected to the greater amount of alcohol (Fig. 6), which were averaging a higher rate of division than the lines treated with the less amount (Fig. 5), were more susceptible to copper sulphate from the beginning than either the non-alcoholized line (Fig. 4) or the line on the less amount of alcohol, and finally died out.

The copper sulphate experiments on the *Stylonychia* culture were carried on for only ten days, but increased susceptibly to copper sulphate is shown by the alcoholized lines (cf. Fig. 7).

The division rate of the three *Paramecium* cultures and the three *Stylonychia* cultures subjected to copper sulphate shows distincly that the organisms which have been treated with alcohol are less resistant to copper sulphate.

VI. General Considerations.

The aim of this paper has been to set forth merely the facts which the experiments have revealed. Several possible causes

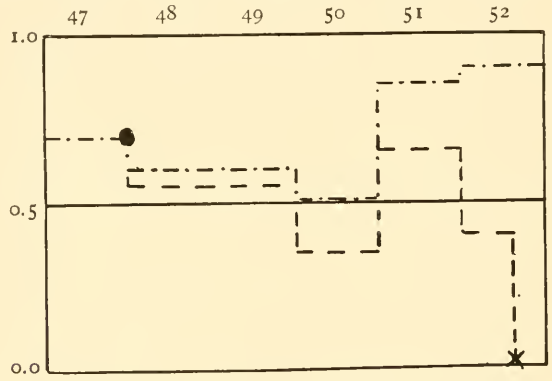

Fig. 5. *Paramecium.* Culture I^2 (hay infusion + alcohol) = · · · · line; culture $I^{\frac{2}{c}}$ (hay infusion + alcohol + $CuSO_4$) = - - - - - line. × = point at which the culture subjected to copper sulphate died out.

of the phenomena observed may be suggested, but it will be of more value to reserve a general discussion of these until more data from experiments are at hand.

Calkins and Lieb, as already mentioned, found that alcohol in medium doses, *c. g.*, one part of alcohol in 2,500 parts of culture medium, acted as a continued stimulus to the division rate of *Paramecium.* The results of my experiments obtained up to the present time fail to show such a marked uniformity of effect from alcohol treatment, as a depression of the rate of division, followed by fluctuations above and below the control, was the result in *Paramecium* I^2 and in all the succeeding cultures of both species, after an initial stimulation of the division rate of a longer

or shorter duration. Since these experiments were conducted with the same general method as that employed by these authors, except that I have carried four lines instead of one line of each alcohol culture and therefore have the average rate of division, the cause of this variation in the results is not apparent. It seems to be clear, however, that alcohol in optimum amounts does usually cause an increase in the rate of division for a certain length of time, and then a falling off of the rate. Whether the

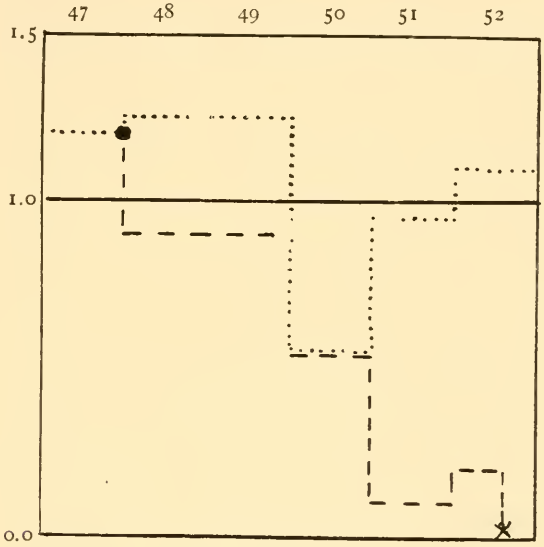

FIG. 6. *Paramecium.* Culture I^{2a} (hay infusion + alcohol) = ····· line; culture I^{2a}_{c} (hay infusion + alcohol + $CuSO_4$) = - - - - - line. × = point at which the culture subjected to copper sulphate died out.

continued stimulation with alcohol will cause a prolongation of the cycle beyond the normal one of the non-alcoholized lines, as found by Calkins and Lieb, remains to be seen.

It is generally accepted at the present time that alcohol has a tendency to prevent the oxidation of other material in the body by its own oxidation — thus alcohol is a "food" rather than a "drug." Without attempting to consider in detail the question of alcohol as a "food" in relation to the metabolism of the protozoa, about which too little is known, I believe that the effect which alcohol exerts on the division rate of infusoria is not to be

interpreted as an increase in the available food supply of the organisms or as a substitute therefor. Any increase in the number of bacteria in the alcohol cultures due to an effect of alcohol on the reproduction of the bacteria can, I believe, be disregarded

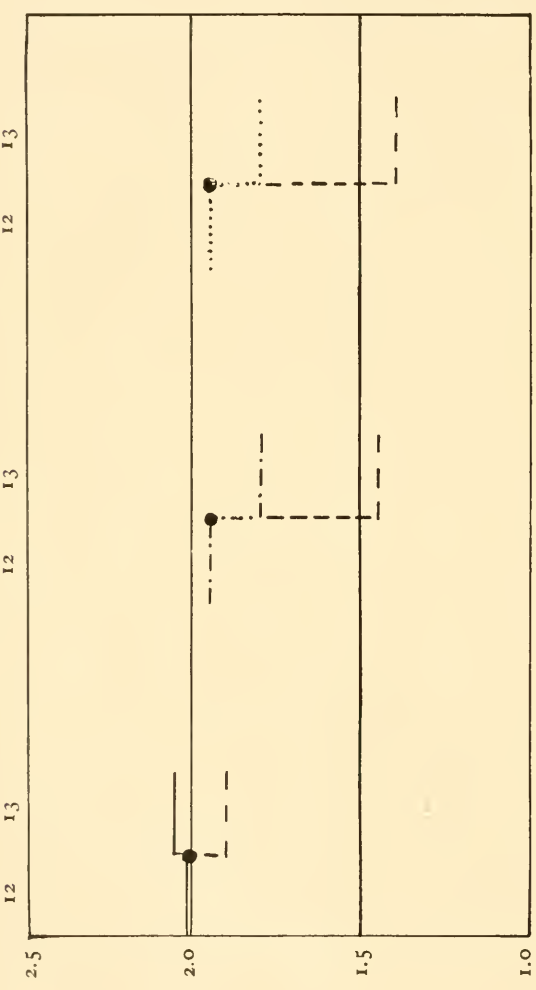

FIG. 7. *Stylonychia.* A. Culture I (hay infusion) = continuous line; culture I_c' subjected to $CuSO_4$ = - - - - line.
B. Culture I^1 (hay infusion + alcohol) = · - · - · line; culture I_c^1 (hay infusion + alcohol + $CuSO_4$) = - - - - line.
C. Culture I^{1a} (hay infusion + alcohol) = · · · · line; culture I_c^{1a} (hay infusion + alcohol + $CuSO_4$) = - - - - line.

because the control cultures show that bacteria develop in the freshly made hay infusion far more rapidly than they are consumed by the animals under the conditions of the experiment. If it be assumed that alcohol serves in a limited sense as a food

in that it is more easily oxidized than the products of the bacteria, and therefore the alcohol-treated lines are able to assimilate more food, I think the fact that the increased division rate of the alcoholized lines is not permanent, but gradually declines and falls below the division rate of the control, suggests that the effect of the alcohol must be more subtle. Again the decrease in the rate of cell division due to alcohol at the early period of the cycle is not so readily explained on the assumption that alcohol is a "food" for the organisms in question.

It is obvious that the movements of the organisms in the culture medium is considerably more rapid, as a rule, when treated with alcohol. This might suggest, since food is largely received through vortex currents passing down the peristome, that more food is thus secured; but I believe that this suggestion is answered negatively by the fact that alcohol does not have a consistently accelerating action.

It might be suspected that an osmotic change brought about by the strength of alcohol used would be sufficient to influence the division rate of the infusoria, but the osmotic change is so exceedingly small that there is no reason to believe, from what is known of the effects of the phenomenon on the cleavage of eggs, etc., that any effect in this case is to be attributed to it.

As far as the experiments go I believe that they indicate that alcohol has a stimulating effect on some aspect of metabolism — possibly, as Calkins has suggested in this connection, on the secreting activities of the protoplasm. I think the evidence derived from the experiments justifies the idea that, in the case of the forms studied, alcohol supplies no "energy," so to speak, but stimulates the liberation of the "initial of potential" with which the organism is endowed.

In other words, we are justified in looking upon the protozoan cell as possessing a certain amount of metabolic energy, or it might be termed "division energy." In the normal course of the cycle, this is gradually expended in reaching the number of generations, more or less, for which the individual is endowed; but when alcohol, for example, is encountered in the environment this tends, directly or indirectly, toward a more rapid liberation of the division energy with the result that

multiplication is more rapid and more generations, for a certain length of time, are produced. But this stimulation of reproduction is not permanent, and in fact the division rate falls temporarily below the normal for the culture, as is shown by the rate of division of the control. Consequently the actual number of generations attained in the cycle is but slightly affected. From this point of view the alcohol has an effect on the individual cell of the cycle — but not on the cycle as a whole. That is, it influences the rate of reproduction but does not affect the number of generations which otherwise would be attained. This assumption will explain possibly the opposite effects produced by alcohol on the cultures at different periods of the cycle. Figs. 1 and 2 show that when the division rate is rapid the alcohol has a general depressing effect — and this may be due to the fact that the maximum division energy is being expended already, whereas when the division rate is on the decline, then the alcohol "stimulates" temporarily, and a greater number of bipartitions occur in a given period, than is the case in the control culture.

It is a point of considerable interest that alcohol produces opposite effects on the division rate at different points in the cycle, and this shows the danger of drawing conclusions from experiments on short cultures or on individuals about the ancestry of which little or nothing is known, which have been isolated merely from stock cultures. The same point is illustrated by some previously published experiments with the salts of potassium [1] — in which it was found that the dibasic potassium phosphate caused an acceleration of the rate of division during the early part of the cycle, and a retardation of the rate during the later part of the cycle. It is to be noted, however, that alcohol caused a retardation of the rate during the early part of the cycle of the *Paramecium aurelia* culture, whereas K_2HPO_4 caused an acceleration of the rate during the early part of the cycle of the *Oxytricha fallax* culture.

To draw any general conclusions from the experiments on copper sulphate at the present time would be hazardous as the results obtained, though definite, are insufficient. The data

[1] Woodruff, *loc. cit.*, pp. 617–619, Diagram IX.

show that organisms which are subjected for long periods to small amounts of alcohol, and which have attained a greater number of generations than the non-alcohol series, are more susceptible to copper sulphate; and that the lines which were subjected to the greater strength of alcohol are more susceptible to the copper sulphate than the series treated with the less strength. This shows clearly that alcohol in such amounts which may be said to be "beneficial" from the standpoint of cell metabolism, since more cell divisions have occurred, nevertheless renders the cells more susceptible to the "injurious" effects of copper sulphate. In what way this is brought about is not evident from the results obtained to date. It seems improbable that we are justified in assuming that the alcohol has caused a general "lowering of resistance" in view of the fact that the general effect of the alcohol is to increase cell division. The results suggest that probably alcohol exerts some specific effect on the metabolism of the organism, or possibly as has been suggested, for example, effects some change in the permeability of the cell membrane to copper sulphate.

VII. Summary.

The experiments briefly recorded were conducted for considerable periods on two species of Protozoa, whose status in the life-cycle was known through long cultures, and on a sufficiently large number of individuals to afford reliable averages. It is believed, therefore, that the results obtained show the general effect of alcohol on the division rate and, therefore, on the metabolism of the forms studied, when subjected to a practically constant environment.

The evidence brought forward shows that:

1. Minute doses of alcohol will decrease the rate of division at one period of the life cycle and increase it at another period of the life cycle.

2. When alcohol increases the division rate, the effect is not continuous, but gradually diminishes and finally the rate of division falls below that of the control, followed by fluctuations above and below the rate of the control.

3. An increase (doubling) of the amount of alcohol adminis-

tered, however, will again cause a more rapid cell division for a limited period. But again the effect is not constant since the rate of division falls below the control, and is followed by fluctuation above and below the division rate of the control. Up to the present time the amount of alcohol has been increased (doubled) three times, always with the same result.

4. Treatment with alcohol lowers the resistance of the organisms to copper sulphate.

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Yale University.

ON THE SPINNING ORGANS AND ARCHITECTURE OF EVAGRUS, A THERAPHOSID ARANEAD.[1]

C. W. STEVENSON.

The most important work on the spinning glands of spiders is that of Apstein (1892), who distinguished the five following kinds:

1. *Aciniform Glands.* — Oval-shaped glands with long duct; gland consists of tunica propria and epithelium, which stains evenly in all parts, duct of which bears no epithelium and ends on a spool with long spinning hair.

2. *Piriform Glands.* — Pear-shaped glands consisting of tunica propria and epithelium, the lower portion of which stains deeper than upper portion; the duct bears a thick tunica intima and ends on a short thick spool, with thick spinning hair.

3. *Ampullaceous Glands.* — Sac-like glands consisting of tunica propria and epithelium, of which the upper portion is cylindrical, then has a sac-like swelling from which the duct consisting of tunica propria, epithelium and tunica intima forms a double loop, the three branches of which are formed in a tunica propria, and ends on a large truncate spinning spool.

4. *Tubuliform Glands.* — Cylindrical glands consisting of tunica propria and epithelium, duct consists of tunica propria, epithelium and tunica intima and ends on a large spool.

5. *Aggregate Glands.* — Aboraceous glands of tunica propria and epithelium, with wide ramifying lumen of which the duct, consisting of tunica propria, epithelium and tunica intima bears protuberances and ends on a large spool with long pointed spinning hair.

Apstein gives only a short description of one of the Theraphosids, *Lasidora Erichsonii*, of the family Aviculariidæ. He found in these only piriform glands, of which the spinning hairs were ringed or annulated.

The only subsequent work is that of Warburton (1890) on Argiopids; McCook (1890) on Epeirids; Borgert's (1890) general

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 92.

review; and descriptions of the external anatomy of the spinnerets given by Simon (1892).

Therefore the spinning glands of Theraphosids are practically unknown and the present contribution is to present an account of a member of that group, and was done under the direction of Prof. Thos. H. Montgomery, Jr.

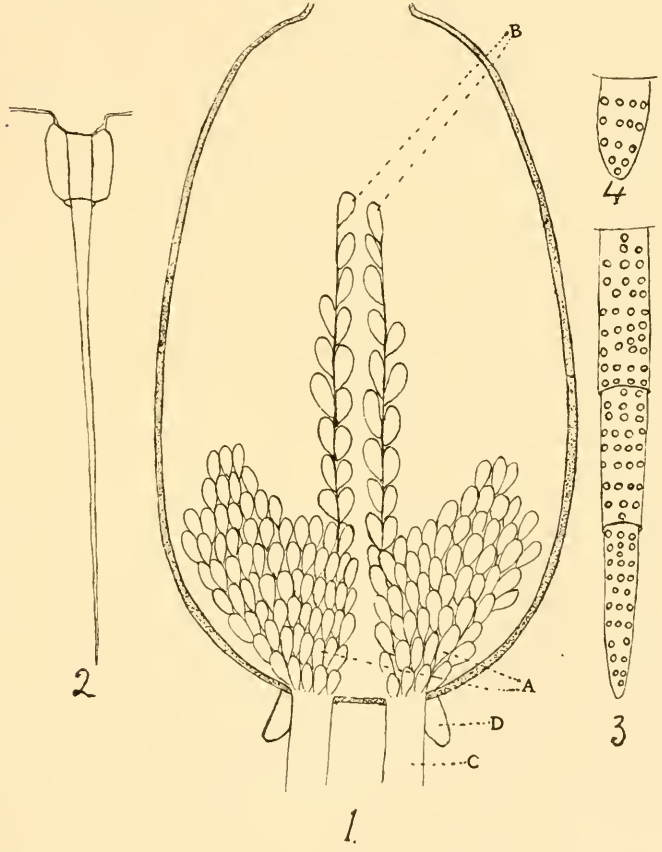
Simon (1892) divides the spiders into *Araneæ Theraphosæ* and *Araneæ Veræ*, the former including all spiders with chelicera directed forward, and comprising the families Liphistiidæ, Avicularidæ and Atypidæ. The Liphistiidæ are unique among all spiders in having four pairs of spinnerets; the Avicularidæ have only two pairs (except *Hexathele* which has three), while the Atypidæ as also most of the families of *Araneæ Veræ*, possess three pairs.

There has been a discussion as to the homologies of the two pairs of spinnerets of the Avicularidæ with the three and four pairs of the Atypidæ and Liphistiidæ. Jaworowski (1895) has shown that in Lycosids the embryonic extremities of the fourth and fifth segments of the abdomen give rise to the spinnerets in the following manner: each extremity of the fourth segment consists of two parts, endopodite and exopodite, of which the exopodites give rise to the anterior pair of spinnerets, while the endopodite of one side fuses with its fellow of the opposite side to form the colulus or its homologue the cribellum. As for the extremities of the fifth segment, the endopodites give rise to the median pair of spinnerets and the exopodites give rise to the posterior pair.

Now, it is possible, as Jaworowski believed, that of the four pairs of spinnerets of the Liphistids, the most anterior pair is homologous with the colulus and cribellum of the *Araneæ Veræ*. But just what are the homologies of the three pairs of spinnerets of the latter to the two pairs of the Avicularidæ cannot yet be decided, as the embryology of no Theraphosid has been worked out, although Simon considers the two pairs of the Avicularidæ to correspond to the anterior and median spinnerets of other spiders, while Jaworowski would hold them as homologous with the anterior and posterior spinnerets of other spiders. Accordingly in calling the spinnerets of *Evagrus* "anterior" and "posterior" respectively, following the general usage, I do not mean to prejudice the question of their homologies.

In my work on *Evagrus*, fresh material was used and the best plan was found to be to dissect them in water or very weak alcohol. The abdomen was opened from the dorsal surface, and the liver, rectum and ovaries removed, leaving the translucent spinning glands exposed to view.

There are only two pairs of glands in both males and females, one pair for each pair of spinnerets, as shown in Fig. 1. The



large pair *a*, which consists of a cluster of about 100 small piriform glands has its outlet through the larger or posterior spinnerets *c*, and the long gland *b* consisting of 12–16 piriform glands, bilaterally arranged, belongs to the smaller or anterior pair of

spinnerets *d*. The ducts of these glands end on the same kind of spools, the spools having three parts, Fig. 2, a short flexible part, then a short heavy chitinous basal piece which terminates in a long spinning hair. According to Apstein's classification, these glands would be piriform glands, being pear-shaped and ending on a spool with short thick base, but differing in having a long spinning hair.

Of the two pair of spinnerets, the posterior consists of three joints, while the anterior has only one short piece. These spinnerets bear spinning spools only on the ventral surface. On the posterior pair, Fig. 3, the spinning spools seem to be about equally distributed over the whole ventral surface, thereby differing from most spiders. On the anterior pair, Fig. 4, the number of spools seem to correspond with the number of glands which empty into it.

The females of two other genera of this same family were dissected, *Stichoplastus* (?) and *Myrmeciophila*, and these were found to agree in general with *Evagrus* in having only two pairs of spinning glands, composed of piriform glands.

In *Stichoplastus*, the glands of the posterior spinneret were similar in position and number to those of *Evagrus* but were a little larger and curved in shape. As to the glands of the anterior spinneret, they were about twelve in number, differing from *Evagrus* in that they varied in size, four being larger and far apart, and eight posterior, small and closer together. In *Myrmeciophila*, the glands were exactly similar to *Evagrus* except in number, *Myrmeciophila* possessing about 25 or 30 glands to the anterior and 150 to 200 to posterior pair of spinnerets.

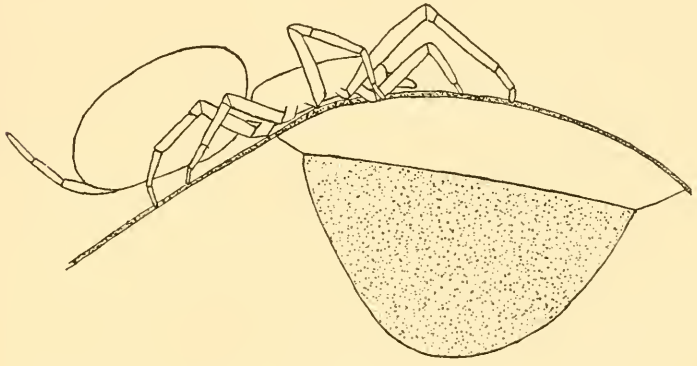
The primitiveness of these Theraphosids is shown in the limb-like elongation of the spinnerets, and in the possession of only two pairs of spinning glands, one pair to each pair of spinnerets, both of which are made up of the same kind of glands.

The only Theraphosids whose architecture has received much attention are the trap-door spiders; accordingly it may have some value to give a brief accout of the web and cocoon of *Evagrus*.

The web is always placed on the ground under large rocks, and generally in shady places where there is moisture. It seems that this species stays in colonies. Often a colony is found in one

place and fifteen feet away not a spider is to be found, from which we may conclude that the young do not scatter much. The web is a very primitive structure, containing no viscid threads and is hardly more than a thin irregular sheet woven on the ground and attached to twigs, leaves or the rock itself. There seems to be no definite form of nest but simply an irregular sheet of threads.

The cocoon of *Evagrus* is somewhat conical or cup-shaped as shown in Fig. 5, which figure also gives the relation of the



5

size of spider to that of cocoon, being a sketch of a spider only a short time after she had finished her cocoon. The cocooning process was seen only once and that time the basal piece had been woven before direct observations began. The base, which was woven first, consists of the cup-shaped lower portion. A short time after it was finished, the spider was closely observed during the remainder of the process. At 2:55 P. M. May 4, the spider placed her epigynum across this base, and discharged from her genital aperture a large yellowish drop of viscid fluid, which remained attached to her for some time, while the ova were dropping into it. At 3:15 P. M. she freed herself from this drop and immediately began to spin the cover. She did this, in which process both pairs of spinnerets were used, by standing with her legs and palpi on the margin of the base, and sweeping her spinnerets from side to side over the egg-mass, never at any

time allowing her body to touch the cocoon. Occasionally, she would change her position or take short rests. At first the threads were loosely woven, but at the latter part of the process they were closely woven. The weaving of the cover lasted from 3:19 P. M. to 3:56 P. M. Then by raising her spinnerets and swinging them from side to side above the cover, she built a kind of outside cover or what seemed later to be a suspensor above the cocoon. This lasted until 4:12 P. M. when she began biting the old connection with the web, leaving it suspended at four corners and by means of the suspensor she had just made. At 5:30 she quit operations, and left the cocoon suspended. The finished cocoon is somewhat hemispherical beneath, the base, while the cover is a flat disc. From this observation, and from observing others kept in cages, and also those found in natural conditions, the cocoon seems to be spun upon a part of the web. In the natural conditions, it is suspended from the under side of the web at about the central portion.

Here, it is seen that the cocoon of *Evagrus* consists of two parts, the base and cover. This goes to uphold the observations of Montgomery (1903), as pointed out by him for several genera, that the cocoons of all spiders are made of two parts, the base and the cover.

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ON THE SPERMATOGENESIS OF THE EARWIG ANISOLABIS MARITIMA.[1]

HARRIET RANDOLPH.

The material for the examination of the germ cells of *Anisolabis maritima* came from a colony of these earwigs at Bryn Mawr. On account of the interest in the behavior of the chromosomes in the germ cells of insects, it seems desirable to add this group to the list of those that have been investigated recently in this country.

The material was preserved in Flemming's stronger fluid or in Gilson's mercuro-nitric solution and stained by Heidenhain's iron-hæmatoxylin method or with thionin.

A preliminary paper on the spermatogenesis of *Forficula auricularia* by Zweiger, '06, which appeared in 1906, contains references to the bibliography of the subject.

In the youngest stages found at Bryn Mawr each cyst contained several spermatogonia. In these cells (Fig. 1) in the resting stage there is a large spherical body which stains like chromatin.

In the equatorial plate of the dividing spermatogonia seen in polar view there are twenty-four chromosomes and a plasmosome which stains faintly (Fig. 2).

In the telophase of the last spermatogonial division two chromosome rods become connected with the plasmosome and remain condensed throughout the growth stages of the first spermatocytes.

Synizesis and synapsis stages are shown in Figs. 3 and 4, and the spireme in Fig. 5. At some time during the growth stages of the spermatocyte the heterochromosome pair separate from the plasmosome, forming a single rounded mass which lies free in the nuclear space (Figs. 6 and 7).

The splitting of the chromosomes is shown in Fig. 8. In Fig. 9 chromosomes from the prophase of the first spermatocyte are

[1] For the identification of the species I am indebted to the kindness of Mr. J. A. G. Rehn, of the Academy of Natural Sciences, Philadelphia, Penna.

shown, together with the heterochromosome pair and the plasmosome. All are from the same cyst. Figs. 10, 11 and 12 are from late prophases of the first spermatocyte division. The chromosomes arrange themselves into two groups at opposite poles. This is shown in Fig. 10, where the black bodies represent one group and those in light outline are 180° away, while the one in heavy outline is approximately at the equator. Figs. 11 and 12 show a centrosome close to each group which had apparently moved with its centrosome to that position. That the final position of any chromosome at one or the other of the poles is due to the centrosome in whose sphere of influence it happens to lie is suggested by the fact that occasionally seven chromosomes are at one pole and only five at the other.

In the equatorial plate of the first spermatocyte division there are normally twelve chromosomes (Fig. 13). In two or three earwigs a few cells of a cyst show eleven, thirteen, sixteen or nineteen chromosomes in the equatorial plate of this division; but there are also present in these cysts tripolar or multipolar spindles which probably explain the irregularity. There are also occasionally giant nuclei with double the normal number of chromosomes. In one case of an abnormal spindle it is known that the material came from an earwig that had very recently moulted, and it is possible that there is a connection between the two facts (Riddle, '08). In one instance a tripolar spindle was observed also in a spermatogonial division.

In the anaphase of the first spermatocyte division the heterochromosome pair are late in dividing and lag behind the others (Figs. 15, 16, 17). They are about equal in size. They finally separate (Figs. 18, 19, 20), one going to each pole of the spindle, and pass into the prophase of the second spermatocyte division (Fig. 21). Here again the chromosomes show a tendency to arrange themselves at the poles. Fig. 22 shows the metaphase and Fig. 23 the equatorial plate of the second spermatocyte division with twelve chromosomes. Figs. 24 and 25 are from stages in the anaphase. The earliest stages of the spermatids are shown in Figs. 26, 27, 28 and 29, where the behavior of the archoplasm and the change in position of the centrosome can be seen. Fig. 32 shows the condensed chromatin body in

the young spermatozoa. The older spermatozoa arrange themselves in bundles by inserting their heads into a cyst cell.

The material which is to form the spindle fibers is conspicuous at an early stage and is very considerable in amount. It forms another layer of fibers around the spindle proper (Figs. 5, 21, 22).

Something like the "mitosoma" described for *Forficula* by Zweiger, '06, is present in *Anisolabis* (Fig. 1), although the form is apparently unlike in the two species. It is traceable possibly from the spermatogonium to the spermatid; but as it does not stain with thionin after an early stage, and as it is very small and there are many granules in the iron-hæmatoxylin preparation, it is not by any means certain that the structures observed are one and the same throughout the series (Fig. 20).

The somatic chromosome number, found in the cells of the egg follicle, is twenty-four (Fig. 30). The material was not favorable for the examination of the ova. Very few were found in division stages and only one was cut so that its chromosomes could be counted. In this only equatorial plate observed the number of the chromosomes is twenty-four.

The characteristic structure of the male germ cells of *Anisolabis maritima* is an equal heterochromosome pair which are present possibly in the spermatogonia although they are not distinguishable from the other chromosomes in the spermatogonial divisions. In any case, it is formed anew in the telophase of the last spermatogonial division. It remains condensed during the growth stages of the first spermatocyte and divides equally in the first spermatocyte division, lagging behind the other chromosomes in the anaphase. It is not evident in the second spermatocyte division but there is a condensed chromatin body in the spermatids. This equal heterochromosome pair appears to be like the equal pair of idiochromosomes found by Wilson, '05, in *Nezara* and the equal heterochromosome pair of Stevens, '06, in Lepidoptera.

Bryn Mawr College,
June 2, 1908.

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Explanation of Plate I.

All figures were drawn with Zeiss camera lucida, 2 mm. oil immersion objective, 12 ocular, enlarged 2 diameters with a drawing camera and reduced the same.

Fig. 1. Spermatogonium, resting stage. *m* (?), the "mitosoma" of Zweiger.

Fig. 2. Spermatogonium, equatorial plate. *p*, the plasmosome.

Fig. 3. First spermatocyte, synizesis stage. *hc*, the heterochromosome.

Fig. 4. First spermatocyte, synapsis stage.

Fig. 5. First spermatocyte, spireme stage.

Fig. 6. First spermatocyte, the heterochromosome pair partially separated from the plasmosome.

Fig. 7. First spermatocyte, the heterochromosome pair separated from the plasmosome.

Fig. 8. First spermatocyte, the chromosomes partially split.

Fig. 9. First spermatocyte, prophase; the forms of the chromosomes, all from the same cyst.

Fig. 10. First spermatocyte, prophase; the six chromosomes in solid black are at one pole and the five in outline at the opposite pole; the one in heavy outline is at the equator.

Figs. 11 and 12. First spermatocyte, prophase; the chromosomes on the way to opposite poles under the influence of the centrosomes.

Fig. 13. First spermatocyte, equatorial plate.

Fig. 14. First spermatocyte, metaphase.

Fig. 15. First spermatocyte, early anaphase.

Figs. 16 and 17. Later anaphase stages.

m
1
2
p
3
h
4
h
5
h
6
h
p
7
p
h
8
p
h
9
p
h
10
11
12
13
14
15
16
17

Explanation of Plate II.

Figs. 18 and 19. Late anaphase stages.

Fig. 20. Telophase; *?m*, the "mitosoma" of Zweiger.

Fig. 21. Second spermatocyte, prophase.

Fig. 22. Second spermatocyte, metaphase.

Fig. 23. Second spermatocyte, equatorial plate.

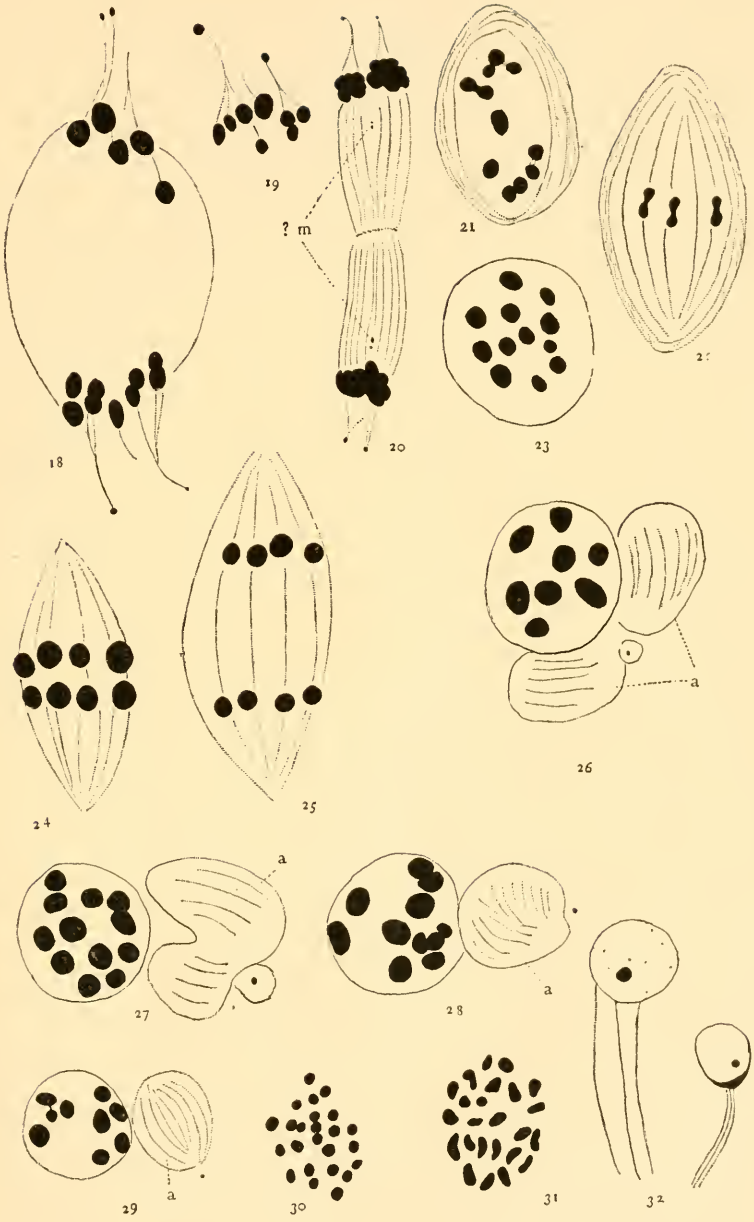
Figs. 24 and 25. Second spermatocyte, anaphase stages.

Figs. 26, 27, 28, 29. Spermatids, showing archoplasma (*a*) in different stages, and the movement of the centrosome.

Fig. 30. Somatic cell of female, equatorial plate.

Fig. 31. Ovum, equatorial plate.

Fig. 32. Young spermatozoa containing condensed chromatin.


19
? m
21
18
20
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a
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a
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a
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31
32

TABLE OF CHARACTERS.

| Species. | Length in mm | Width in Per Cent. of Length | Lat. Field. Large | Lat. Field. Small | Muscle. Strong | Muscle. Weak | Diameter of Septum. Broad | Diameter of Septum. Narrow | Position of Greatest Width. Sudden | Position of Greatest Width. Gradual | Position of Greatest Width. None | Position of Greatest Width. At Center. | Tail. Behind Center | Tail. In Front Center | Tail. Extends Wide | Tail Length in Per Cent. of Total Length. | Lateral Sensitive Tubercles. On Head | Lateral Sensitive Tubercles. On Body. | Lateral Sensitive Tubercles. On Both | General Appearance. Collector Present | General Appearance. Collector Absent | General Appearance. Opaque. | General Appearance. Transparent. | General Appearance. Firm | General Appearance. Flaccid | Anterior Fin. Number of Anterior Teeth | Anterior Fin. Number of Posterior Teeth | Anterior Fin. Length in Per Cent of Total Length. | Anterior Fin. Width in Per Cent. of Length. | Anterior Fin. Extends to Ventral Ganglion. | Anterior Fin. Extends to Second Ventral Ganglion. | Anterior Fin. Approaching Ventral Ganglion. | Anterior Fin. Widest at Center. | Anterior Fin. Widest Behind Center. | Anterior Fin. Widest Before Center. | Anterior Fin. Distance from Anterior to Posterior Fins in Per Cent of Total Length. | Posterior Fin. Length in Per Cent. of Total Length. | Posterior Fin. Width in Per Cent. of Total Length. | Posterior Fin. Extends to Ventral Ganglion. | Posterior Fin. Not to Ventral Ganglion. | Posterior Fin. Longer Than Anterior Fin. | Posterior Fin. Shorter Than Anterior Fin. | Posterior Fin. Same as Anterior Fin. | Posterior Fin. Widest at Tail Septum. | Posterior Fin. Widest in Front of Tail Septum. | Posterior Fin. Widest Behind Tail Septum. | Ventral or Ridge. More Than 50 Per Cent in Front of Tail Septum. | Ventral or Ridge. Less Than 50 Per Cent in Front of Tail Septum. | Ventral or Ridge. Teeth Row Longer | Ventral or Ridge. Teeth Row Shorter | Ventral or Ridge. Lateral Process Blunt. | Ventral or Ridge. Lateral Process Acute. | Ventral or Ridge. Acute Papillæ. | Ventral or Ridge. Plate Papillæ. | Shaft. Number. | Shaft. Greatly Curved. | Shaft. Slightly Curved. | Shaft. Not Serrated. | Shaft. Serrated. | Shaft. Not Crested. | Shaft. Crested. | Shaft. Cross-section Oval. | Shaft. Cross-section Triangular. | Shaft. Pes Shaped. | Shaft. Slightly Curved at Apex. | Shaft. Broadly Curved. | Hooks. Point. So Shaped. | Hooks. Point. Per Cent. on the Shaft. | Hooks. Base and Top of Joint. Coverage at Base. | Hooks. Base and Top of Joint. Divergence at Base. | Hooks. Base and Top of Joint. Divergence at Top. | Hooks. Base and Top of Joint. At Focus. | Hooks. Base and Top of Joint. At Center. | Hooks. Base and Top of Joint. Between the Two. | Pes. Smaller Near Base of Pinna. | Pes. Base Greater at Point. | Pes. Strength. | Pes. Laud Scattered. | Pes. Laud Concentrated. |
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Vol. XV. *August, 1908.* *No. 3.*

BIOLOGICAL BULLETIN

THE SEGREGATION OF THE GERM-CELLS OF PHRYNOSOMA CORNUTUM: PRELIMINARY NOTE.[1]

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The changes that have been effected in the theories of germ-cell origin since 1870 are characteristic of the advance of scientific thought in general. Waldeyer's theory (1870) of their origin from peritoneal cells was, in its very simplicity, so attractive that it received universal recognition; and since Nussbaum's opposing theory (1880) that the germ-cells are to be observed in the late cleavage stages of the egg, there has been continual controversy over every new detail. The phenomenon of migration of the germ-cells was observed in sponges long before Nussbaum; Balfour's evidence (1878) indicates a migration from the mesentery to the genital ridges, and Balfour admits the possibility of a migration from the blastoderm; Weismann proved conclusively its occurrence in the hydroid polyps; and yet modern embryologists seem slow to admit that the primitive germ-cells in vertebrates have the power of independent movement.

Eigenmann (1891) was the first to give a detailed account of the wanderings of the germ-cells in vertebrates; he described the migration from before backward in *Cymatogaster* (*Micrometrus*), and in 1896, a dorso-median migration in this teleost. Hoffmann (1892) noted a probable migration from the connective tissue between splanchnic mesoblast and entoblast to the germinal epithelium, and Rabl (1896) gave a detailed description of their very general distribution in selachians. It would seem that

[1] Contributions from the Zoölogical Laboratory of The University of Texas, No. 93.

Wheeler's (1899) work on the lamprey is conclusive evidence; he found the germ-cells first in the entoblast lateral to the myotome, in the mesoblast after it is cut off, and then moving into the median line. Bouin (1900) argues that the germ-cells arise directly from the peritoneal and sclerotome cells of the germinal region, but admits that they may also come from the yolk-sac. Nussbaum (1901) records their migration in *Gallus* from the splanchnic mesoblast, where they appear as large, yolk-laden cells, the ova being their descendants. Woods (1902) gives an interesting account of the germ-cells in *Acanthias*, where they first appear in the entoblast or in the yolk, migrating to the germ-gland anlagen.

Beard (1902) studied the germ-cells in various selachians. In *Raja* he traced them back to late cleavage stages; many come to lie in the germinal cavity, whence they migrate into the embryo, and along the space between entoblast and mesoblast to the germinal nidus; some leave this normal path and reach other organs of the embryo. He considers that some of these latter degenerate.

Böhi (1904) found that the first germ-cells appear in the trout-embryo not before the twenty-fifth day after fertilization; in the salmon, not before the thirty-first. They lie in the splanchnic and somatic mesoblast, being pushed into the somatic by the growth of the splanchnic.

Allen (1906) working on *Chrysemys*, found that the germ-cells originate in the entoblast at the edge of the area pellucida, in a zone extending from a point opposite the anterior end of the pronephros to a point behind the embryo. They migrate in the entoblast to a point beneath the notochord, and upward through the sclerotome to the germ-gland anlagen. A part of these also "lose the way." In *Rana pipiens* (1907) he finds that they develop from a group of apparently indifferent entoblast cells in the dorso-median region; this group is cut off from the other entoblast cells by the growth of the mesentery, or more probably, moves above the mesentery; later, the cells migrate laterally coming to lie in the paired germ-gland anlagen.

Through the courtesy of Dr. Allen, I have been enabled to examine the more important stages in the migration of the germ-cells of *Chrysemys;* they are similar to my own material, as my

conclusions, although differing from Dr. Allen's in details of early distribution and period of migration, uphold his.

My work was done on *Phrynosoma cornutum* Harlan, of central Texas. The material was largely supplied from the collections of Miss Augusta Rucker; I take this opportunity to express my thanks to her, and also to Dr. Thomas H. Montgomery, under whose direction this study was completed.

The embryos were fixed in nitric acid, Zenker's fluid, and picro-sulphuric acid, the last proving to be the most satisfactory; the sections were double stained with Heidenhain's iron hæmatoxylin and alcoholic eosin, and sections were made 6 micra thick.

The most careful study was given to series No. 26; a stage where the intestine is completely closed except at the region of the yolk-stalk which is 1.5 mm. wide; the allantois is well developed, and two visceral clefts have broken through. This was compared with series No. 12, an embryo with five visceral clefts. Each section was examined under an immersion lens, and the number and positions of the germ-cells were tabulated. In series No. 12, the peritoneum of the germ-gland anlagen is distinctly thickened throughout the region of the mesonephros; within these thickenings there are found at intervals large rounded cells, with clear cytoplasm containing yolk and large clear nuclei with deep-staining chromatin granules. Two of these are drawn in Fig. 1; the nucleus of the left germ-cell measures 6 micra and 5 micra in its diameters; that of the right, 7 micra and 6 micra. These were called typical germ-cells; considering their appearance and position, no other satisfactory explanation of their nature

		Series 12.	Series 26.	Series 27.
Sclerotome	lateral to central nervous system		4	one row of sec.
	lateral to aorta	9	1	
	within mesentery	22	5	7
	of yolk-stalk	4		2
Mesothelium		3	4	
Germinal anlage		44	2	2
Ectoblast			1	
Entoblast	of axial intestine	8		
	of yolk-stalk	6		6
Cavity of yolk-stalk		2		
Total		98	17	17

can be given. Their characters were taken as criteria in studying the germ-cells occurring elsewhere.

Similar cells are observed in various regions of the embryo. The above table shows their distribution in series 12 and 26 and one row of sections of series 27, an embryo with three vis-

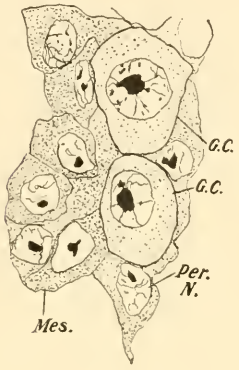

FIG. 1.

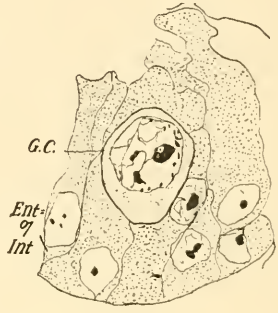

FIG. 2.

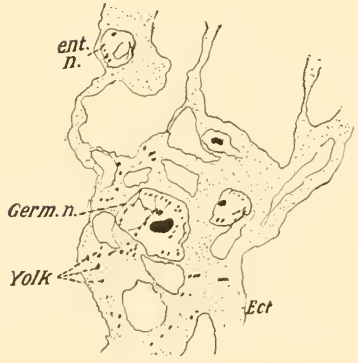

FIG. 3.

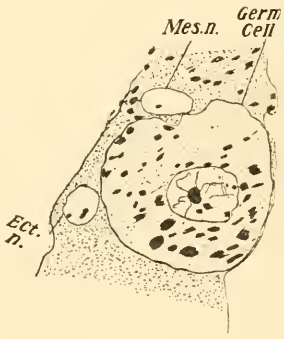

FIG. 4.

ceral clefts, only the row of sections cutting the yolk-stalk being studied.

The germ-cells in the intestinal wall (see Fig. 2), are near the region of the yolk-stalk, a point to be noticed in considering the probable path of migration.

The germ-cells in No. 26 near the central nervous system are interesting, from their location in the anterior portion of the

embryo; one is lateral to the prosencephalon, one to the diencephalon, and one to the mesencephalon, the fourth being in the region of the spinal cord. Also worthy of note are the two within the cavity of the yolk-stalk in series 12, and a third, in the section preceding, which lies partly in the entoblast, partly in the cavity of the yolk-stalk, into which it is apparently migrating.

The figures in series 12 have only comparative, not absolute, value, owing to the facts that some sections were folded so that it was impossible to determine whether or not they contained germ-cells, and that I counted only those cells the nature of which is indisputable. An exact count would undoubtedly give a larger total than 98; but this, when compared with the total of 17 in the younger stage No. 26, requires some explanation of the increase in number.

Mitoses, although frequent among the somatic cells, were not observed among the germ-cells, indicating the period of rest for the germ-cells observed in other vertebrates, and excluding the explanation of the increase in number of germ-cells by division. Of the three other possible explanations, namely the transformation of somatic cells, or of undifferentiated cells within the embryo, into germ-cells, and the migration of germ-cells from the extra-embryonic region into the embryo, the last seems to be the correct one; because, first, no "transitional cells" were observed, and, second, because germ-cells were found in the blastoderm, and in various positions indicating a migration thence into the embryo.

Practically every section of the area vasculosa in series No. 26 contains germ-cells in the entoblast, cephalad, caudad, and laterad of the embryo. I have not counted them in the entire series, but there are several in each section examined, and must be very numerous. These cells have all the characteristics of germ-cells, the nuclei of the surrounding entoblast, mesoblast, and ectoblast cells being distinctly smaller, as shown in Figs. 3 and 4. The drawings do not show so clearly as I could wish that the germ-cells lie in the entoblast rather than in the mesoblast; in fact, their size makes the question difficult to determine, especially since entoblast and mesoblast are not very clearly differentiated; but the nuclei, and often the entire cells, lie below the level of

the blood-vessels, and the lower wall of the cells, where distinguishable, touches the boundary of the entoblast, so that I conclude that they lie normally in the entoblast.

No germ-cells were observed in the extra-embryonic blastoderm of No. 12; in series No. 15, an embryo of the same age, of which about one half the sections were examined in detail, one germ-cell was found in the blastoderm; they were present in the blastoderm of series No. 27.

The path of normal migration is the entoblast of blastoderm, yolk-stalk, and intestine, and the sclerotome of the mesentery to the germinal anlagen; the only other possible path is the mesoblast of the same structures, and the following table shows that the first is the normal path:

	Series 12.	Series 26	Series 27.
Ento. of blastoderm	0	numerous	numerous
Ento. of yolk-stalk	6		6
Ento. of intestine	8		2
Scler. between intestine and germ anlage.	22	9	7
Total in path of normal migration	36	9	15
In meso. of yolk-stalk	4	0	2

Those cells that "lose the way" are found in the various positions tabulated above. It will be observed that the migration is just beginning in series 26, and practically completed in No. 12.

This controverts the suggestion that the cells are passively carried by the concrescence of the germ-layers, since this growth is too slight, between the stages studied, to account for the transposition of the germ-cells.

Now the total number of germ-cells in the older embryo, even allowing for the folded sections, is not nearly equal to the total number of extra-embryonic germ-cells in the younger embryo. The most obvious explanation of the deficit is Beard's theory of degeneration. My observations appear to uphold Beard; I find cells resembling germ-cells in every respect except the size of the nuclei, these being about equal to the somatic nuclei, or apparently absent entirely. They are especially numerous in the sclerotome around the aorta, where the abnormally placed cells are also most numerous. They occur in the cavity of the yolk-stalk, and apparently in the intestinal lumen; the presence of

two indubitable germ-cells in the cavity of the yolk-stalk, and of a third half imbedded in the entoblast and half projecting into the same cavity, lends further support to the theory. However, it will require an exact enumeration of the blastodermic and embryonic germ-cells of many embryos at various stages, as well as work along other lines, to determine the question in *Phrynosoma*. The possibility that these might be transitional forms between somatic and germ-cells is precluded, it seems to me, by the fact that they are not found in what I have termed the path of normal migration of the germ-cells, and especially by their non-appearance in the germinal anlagen.

Summary.

1. The germ-cells appear first in the entoblast of the vascular area of the blastoderm. They lie cephalad, caudad, and laterad to the embryo.

2. The germ-cells migrate in the entoblast of the blastoderm, yolk-stalk, and intestine, and the sclerotome of the mesentery, to the germinal anlagen. Very many leave this path and come to lie in various regions of the embryo.

3. Some of these abnormally placed germ-cells probably degenerate.

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THE CHROMOSOMES IN CROSS-FERTILIZED ECHINOID EGGS.

D. H. TENNENT.

In experiments which I carried on during the summer of 1907[1] crosses were made between several echinoids, namely, by the fertilization of:

1. The egg of the spatangoid *Moira atropos* with the sperm of the sand-dollar *Mellita pentapora*.
2. The egg of *Moira* with the sperm of the sea urchin *Toxopneustes variegatus*.
3. The egg of *Moira* with the sperm of the sea urchin *Arbacia punctulata*.
4. The egg of *Toxopneustes* with the sperm of *Moira*.
5. The egg of *Toxopneustes* with the sperm of *Mellita*.
6. The egg of *Arbacia* with the sperm of *Moira*.
7. The egg of *Arbacia* with the sperm of *Mellita*.
8. The egg of *Mellita* with the sperm of *Moira*.

The work was undertaken primarily with the object of obtaining material for a cytological study of cross-fertilized eggs and secondarily for the purpose of making a comparison, based especially upon the character of the skeleton, between larval forms.

In this paper I shall consider some of the earlier phenomena exhibited, in brief, the prophases and early metaphases of division, in two of the crosses, (1), $\frac{Moira\ ♂}{Toxopneustes\ ♀}$ and (2), $\frac{Moira\ ♂}{Arbacia\ ♀}$, reserving the consideration of later stages, of the other crosses, and a general discussion of the results for a latter contribution.

The method of effecting the cross-fertilization was the exceedingly simple one of allowing the eggs, after their removal from the ovary, to stand for several hours in sea water, the water being changed occasionally, and at the most favorable time, which was

[1] I wish to express my thanks to the Hon. George M. Bowers, U. S. Commissioner of Fisheries, for the privilege of working in the Beaufort Laboratory and to Mr Henry D. Aller, director of the laboratory, for many courtesies extended to me. I am also indebted to Dr. Bartgis McGlone for information regarding the artificial fertilization of *Moira* eggs.

determined by experiment, to fertilize the eggs with normally very active sperm.

All attempts at cross-fertilization of the eggs immediately after their removal from the ovary were unsuccessful.

For the $\frac{\textit{Moira}\ ♂}{\textit{Toxopneustes}\ ♀}$ crosses the eggs were allowed to stand in sea water for five hours and for the $\frac{\textit{Moira}\ ♂}{\textit{Arbacia}\ ♀}$ crosses for seven hours, before fertilization.

Fully 95 per cent. of the eggs so treated underwent a regular and comparatively uniform cleavage, the greater number developing into swimming blastulæ and gastrulæ. About 75 per cent. of these embryos never developed beyond this stage. The remaining 25 per cent. developed into plutei which remained alive and were kept under observation for about ten days.

This high percentage of segmentation was never approached in experiments in which chemicals were employed as aids in effecting cross-fertilization, although naturally no attempts to bring such methods to perfection were made after I had obtained so simple a means of bringing about the results that I desired.

The fact that the eggs were actually fertilized was recognized in the transparent *Toxopneustes* eggs by the observation of the union of the pronuclei. In the cases of both the *Toxopneustes* and *Arbacia* eggs a fertilization membrane was formed.

Each series was checked by a control series of unfertilized eggs. In these controls the eggs were allowed to stand, with occasional changes of sea water, and in every instance the eggs ultimately disintegrated without undergoing segmentation.

The figures that illustrate the account that follows were drawn from sections of picro-acetic and sublimate-acetic material stained in iron hæmatoxylin.

A. The *Moira* ♂ *Toxopneustes* ♀ Cross.

The *Toxopneustes* eggs stood in sea water, which was changed four times, for five hours when they were fertilized with *Moira* sperm.

Cleavage began 40–45 minutes later. The time consumed during the entrance of the spermatozoön, fusion of the pronu-

clei, formation of the amphiaster, etc., is then approximately the same as in *Toxopneustes* eggs fertilized with *Toxopneustes* sperm.

The chromosomes as seen in a polar view of the equatorial plate of $\frac{\textit{Toxopneustes}\ ♂}{\textit{Toxopneustes}\ ♀}$ eggs are shown in Figs. 1 and 2.

They are seen to have the appearance of rather long, slender, and somewhat bent rods. By comparing these two figures it may be seen that variations in the form of the chromosomes, which are correlated with slight differences in the ages of the plates, are evident.

A corresponding view of the chromosomes in a section of a $\frac{\textit{Moira}\ ♂}{\textit{Moira}\ ♀}$ egg is shown in Fig. 3.

Some differences are apparent, but in general the size, form, etc., of the chromosomes in this plate are so like those of the *Toxopneustes* egg that one need scarcely venture to hope to be able to identify the chromosomes of maternal and paternal origin in the cross-fertilized eggs.

An examination of sections such as are illustrated in Figs. 4 and 5 convinces me that we have here a mixture of the two sorts, but I find myself unwilling or perhaps unable to distinguish the chromosomes of either origin.

Some interesting variations from the normal were found in one series of $\frac{\textit{Moira}\ ♂}{\textit{Toxopneustes}\ ♀}$ eggs in which more than one spermatozoön had entered the egg. Two different results are shown in Figs. 9–12 and Text Fig. 1.

In one case the extra sperm-nucleus is seen moving toward the segmentation nucleus. Its aster has divided while the centrosome of the future cleavage amphiaster is still single (Fig. 9). Later the second sperm nucleus seems about to fuse with the segmentation nucleus while the centers of the regular cleavage amphiaster have separated (Fig. 10). In some cases fusion between the two nuclei takes place; in others (Fig. 11), the fibers from one of the sperm asters enter the nucleus and the chromosomes become differentiated in the network before the cleavage asters have well separated.

In the other case (Fig. 12 and Text Fig. 1), chromosomal

differentiation and separation of the cleavage centers had gone on to a considerable extent before the additional spermatozoön had entered the egg. Here the two amphiasters are seen side by side. In Text Fig. 1 what may possibly be sperm tails are seen lying within a fertilization cone, although the entrance of the tail

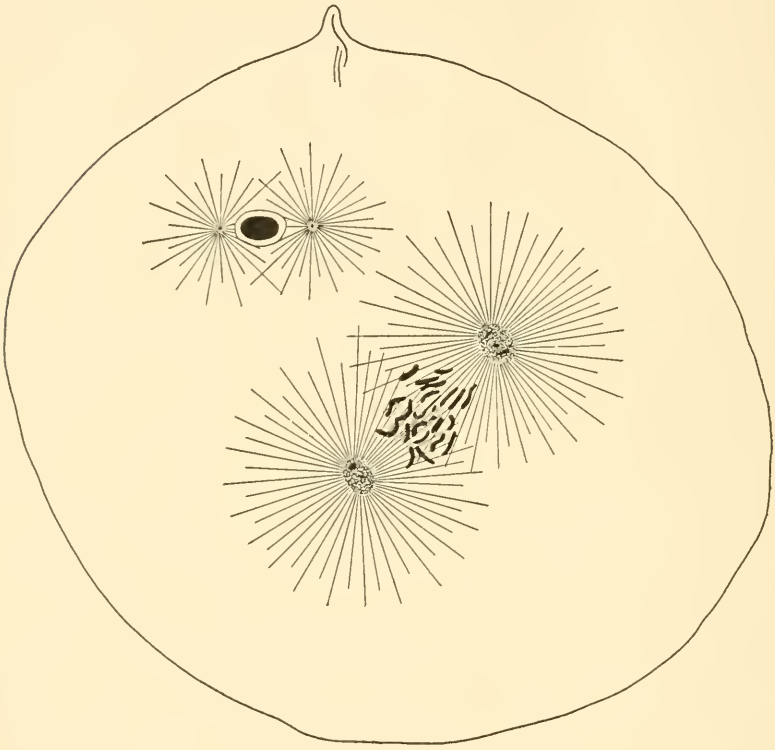


FIG. 1. *Toxopneustes* egg × *Moira* sperm. (Drawn to same scale and reduced slightly more than are plate figures.) Segmentation nucleus dividing. Extra sperm nucleus in prophase.

in the fertilization of the echinoderm egg is contrary to the general belief.

These cases ought to prove of interest in further investigation along the lines laid down by Boveri in his recent contribution on dispermic sea urchin eggs (Zellen-Studien, Heft 6).

B. The *Moira* ♂ *Arbacia* ♀ Cross.

In effecting this cross the *Arbacia* eggs were allowed to stand in sea water for seven hours, the water being changed every hour, and then fertilized. The controls gave no segmentation.

Cleavage began about forty minutes later; again approximately as in normally fertilized eggs, in both cases being slightly hastened or retarded by variations in the temperature of the water.

Fig. 2. *Arbacia* egg × *Moira* sperm. Egg outline omitted, otherwise drawn to same scale and reduced as are plate figures. Chromosomes scattered throughout cytoplasm. *Arbacia* chromosomes and *Moira* chromosomes may be distinguished from one another by size.

The sections of eggs of this cross are perhaps of greater interest than those of the *Moira-Toxopneustes* cross because of the fact that the chromosomes of the two species are of sufficient difference in form to be distinguished from one another.

Fig. 6 shows the chromosomes of an equatorial plate of an $\frac{Arbacia\ \delta}{Arbacia\ \varphi}$ egg. The chromosomes here are seen to be short, slightly bent rods. These are quite different in form from those already mentioned in the equatorial plate of *Moira* (Fig. 3), where the chromosomes are longer and comparatively more slender.

The sections of the *Moira-Arbacia* cross-fertilized eggs giving a polar view of the equatorial plates (Figs. 7 and 8), show a mixture of short and long forms probably indicating *Arbacia* and *Moira* chromosomes respectively. These differences in form are evident in the equatorial plates of both the first and second cleavages, which is as far as I have carried the observations.

The differences in form are less evident in the late metaphases or early anaphases when the daughter chromosomes are drawn out, behaving like substances with a high surface tension, and then contracting during the late anaphases, into much shorter rods.

In both of the crosses, but especially in sections of eggs of the *Moira-Arbacia* cross, an interesting phenomenon may be noted (Text Fig. 2).

In eggs in which the daughter nuclei are in the resting condition succeeding the first division, the cytoplasm contains many deeply staining rods. The nucleus at this time does not take the chromatin stain and appears like an empty vesicular structure.

In eggs, of the same lot and on the same slides, in which the fibers of the second amphiaster have begun to form, the nucleus again takes the stain and shows the chromatic net, while the cytoplasm is seen to be free from the bodies described.

These structures have puzzled me not a little, but I have finally reached the conclusion that the eggs in which they occur are degenerating. Even though this be true it is difficult to explain the simulation or perhaps occurrence of longitudinal and transverse divisions of these chromosomes lying free in the cytoplasm.

Summary.

This paper deals with observations made on sections of cross-fertilized eggs of two kinds: (1) *Toxopneustes* eggs fertilized with *Moira* sperm, (2) *Arbacia* eggs fertilized with *Moira* sperm.

The results of the study may be summarized as follows :

1. The equatorial plate of the *Moira-Toxopneustes* cross shows a mixture of two kinds of chromosomes not sufficiently unlike one another to enable a positive distinction between the two.

2. The equatorial plate of the *Moira-Arbacia* cross shows a mixture of two kinds of chromosomes, one variety long, the other variety short. These differences in form are correlated with the spermatozoön and the egg respectively.

Bryn Mawr College,
March, 1908.

Explanation of Plate I.

All of the figures were drawn with the aid of a camera and Zeiss compensation ocular 12 and 2 mm. Apochromatic oil immersion objective. They were enlarged two diameters with a drawing camera and have been reduced to one half in reproduction.

Fig. 1. *Toxopneustes* egg × *Toxopneustes* sperm. Equatorial plate. Polar view.

Fig. 2. Same as Fig. 1.

Fig. 3. *Moira* egg × *Moira* sperm. Eq. pl.

Fig. 4. *Toxopneustes* egg × *Moira* sperm. Eq. pl.

Fig. 5. Same as Fig. 4.

Fig. 6. *Arbacia* egg × *Arbacia* sperm. Eq. pl.

Fig. 7. *Arbacia* egg × *Moira* sperm. Eq. pl.

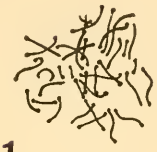
Fig. 8. Same as Fig. 7.

Fig. 9. *Toxopneustes* egg × *Moira* sperm. Segmentation nucleus with centrosome undivided. Extra sperm nucleus with aster divided.

Fig. 10. *Toxopneustes* egg × *Moira* sperm. Segmentation nucleus with centrosome divided. Extra male nucleus, with its aster divided, in contact with segmentation nucleus.

Fig. 11. *Toxopneustes* egg × *Moira* sperm. Segmentation nucleus with centrosome divided. Fibers from aster of extra sperm nucleus extending into the segmentation nucleus.

Fig. 12. *Toxopneustes* egg × *Moira* sperm. Segmentation nucleus and extra sperm nucleus lying side by side and both preparing for division.



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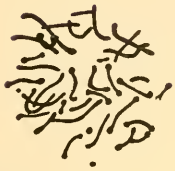

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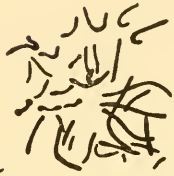

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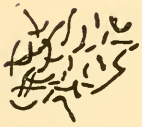

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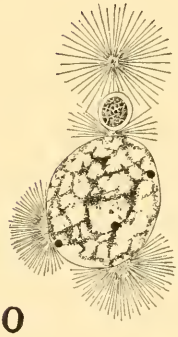

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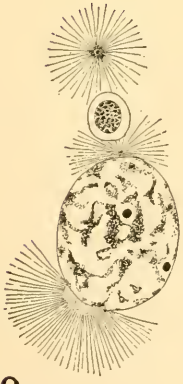

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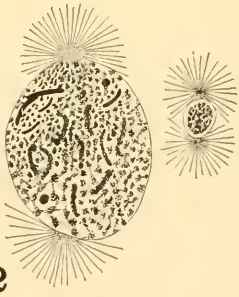

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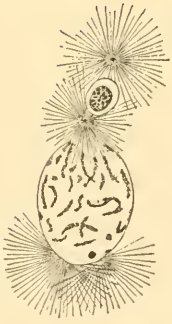

11

SOME HABITS AND SENSORY ADAPTATIONS OF CAVE-INHABITING BATS.[1]

WALTER LOUIS HAHN.

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General Introduction.

The present paper embodies the results of about two years of observation on the habits of bats in caves and in the laboratory. The subjects to which special attention has been paid are: The choice of a dwelling; the factors determining times of activity

[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 95, being a thesis accepted as in part fulfilling the requirements for the degree of Doctor of Philosophy.

and rest; feeding habits; breeding habits; and locomotion, including the sense of direction and means of avoiding obstacles. The senses of direction and means of avoiding obstacles have been investigated experimentally. Studies on the other topics have been carried on largely by observation on free and captive animals.

The work was prosecuted from September 20, 1906, to September 7, 1907, while the author held the Speleological Fellowship in Indiana University with residence at the University's Cave Farm three miles east of Mitchell, Indiana. Later the work was continued in the laboratory of the University at Bloomington, Indiana. There are several caves in the vicinity of both places which are inhabited by a large number of bats, thus affording exceptional opportunities for the study.

Some of the notes, especially those on breeding habits, are very brief. However, it is thought best to include them, together with such facts as those contained in the section on morphological peculiarities, in order to give a more complete idea of the biology of the animals. The experimental studies also need to be continued.

The data presented are in part psychological, but it is the purpose of the present paper to treat it from a biological rather than a psychological standpoint.

The work has been carried on under the direction of Dr. C. H. Eigenmann, professor of zoölogy in Indiana University, to whom I am indebted for constant advice and criticism. My thanks are also due to Dr. Charles Zeleny, associate professor of zoölogy, for helpful suggestions and for aid in revising the manuscript.

Previous Work.

Published observations on the habits of North American Bats are limited to scattered paragraphs in natural histories and taxonomic papers. A number of short papers have been published on English, and a few on Continental European bats. The only extensive studies on the subject are two by Rollinat and Trouessart, the first on the reproduction of the Murine (*Vespertilio murinus*) in 1896, and the second on the sense of direction, in 1900.

Morphological Peculiarities and Relationships.

Bats, constituting the order Chiroptera, are more sharply marked off from their nearest relatives than any other group of mammals. Their closest affinities are with the order insectivora which includes such animals as the moles and shrews. However, the separation is a wide one and no known fossils are in any way intermediate between the two orders.

The most important modification is the adaptation to flight. The changes in structure correlated with the habit of aerial locomotion are the following: The fore limb and pectoral bones and muscles are increased in size and the hind limb and pelvis are reduced. The axis of the hind limb is rotated so that the knee projects backward instead of forward. The digits of the fore limb are lengthened to form a support for the wing membrane. A thin, flexible membrane extends from the sides of the body to the tips of the fingers and from in front of the fore arm to the hind limbs and usually includes the space between the latter and the tail.

Other structural peculiarities are as follows: The carpus is reduced or almost wanting. The first digit (thumb) of the manus is short, nearly free from the wing membrane, opposable, and terminated by a curved claw. The other digits of the manus are long, slender, included in the wing membrane and not terminated by nail or claw. The pes has five short, subequal digits, each with a curved claw. The mammæ are pectoral and there is usually but one pair, although a few species have two pairs. The cerebral lobes are without convolutions, and the cerebellum is relatively large. The ear conch has a slender internal lobule called the tragus in most species, and in several families there are foliaceous appendages of skin about the nostrils.

These structural modifications are worthy of note because they are correlated with the characteristic habits of the animals. Walking or running, after the manner of most animals, is seriously impeded by the lengthening of the fingers, the presence of a membrane joining the limbs, and by the reversal of the direction of the knee flexure. Locomotion on solid surfaces is therefore the rare exception and flight is the common method of progression.

The hind limbs are of use chiefly for clinging while at rest; the fore limbs form only an inadequate support for the animal while at rest and they cannot be used at all for grasping as in most mammals but, as in birds, they are the chief organs of locomotion.

Most insectivorous and carnivorous mammals use the paws to assist in seizing and killing prey and, at times, rest their food against some solid object while eating it. Bats, on the contrary, seize their prey with the mouth, like swallows and flycatchers, and the large, mobile lips assist in holding the food and drawing it into the mouth. They usually masticate it while flying and do not recover any portion that may be dropped.

So greatly has this method of feeding modified the habits of our common vespertilionine bats that the caged animals rarely learn to take food from a dish or from the floor of the cage, although they will eat it readily if it is held directly in front of them.

The expansion of the integument to form the flying membranes has furnished additional surface for bearing organs of special sense and according to Schöbl ('71) a large number of tactile organs are found in the skin of the wing membranes. The nasal appendages and the tragus also have a sensory function, the exact nature of which is not clearly understood.

The nocturnal or crepuscular habit, which is shared by all bats, is doubtless correlated with the increased number of sense organs in the skin which makes the eyes of less importance to the individual and enables it to be active in the absence of light.

Throughout the order there is a relative uniformity of both habit and form. A few species have white markings. In many others the ventral side of the body is paler than the dorsal but otherwise there is a great uniformity of coloration, the prevailing color being some shade of brown. I have seen almost as much variation in the color of a single species from a restricted area as there is in the entire order. While the details of tooth and skeletal structure show that not all the members of the order are closely related, yet the external forms of widely separated groups resemble each other more closely than they do in some of the more nearly related species of other orders.

The Species Studied.

All of the bats found in the United States, except a few species along the southern border, belong to the typical family Vespertilionidæ. In the caves of southern Indiana six species belonging to four genera are found living more or less commonly. In order of greatest abundance they are: The little brown bat, *Myotis lucifugus;* the Georgian bat, *Pipistrellus subflavus;* the Say bat, *Myotis subulatus;* the large brown bat, *Eptesicus fuscus* (*Vespertilio fuscus* of most recent authors); the big eared bat, *Corynorhinus macrotis;* and the large winged bat, *Myotis velifer.* In the literature on the caves of this region, as well as in some of the faunal papers, these species are hopelessly confused. Since the vernacular name, little brown bat, is frequently applied to all of the species except the big eared and large bats, it seems advisable to use the equally convenient scientific names throughout this paper. The observations have been made chiefly on *Myotis lucifugus* but also to a considerable extent on *Myotis subulatus* and *Pipistrellus subflavus.*

These two species of *Myotis* differ chiefly in the size of the ears, the size and shape of the tragus, and some details of cranial structure and dentition. They are about the same size and have the same general appearance and essentially the same habits. *M. lucifugus* is much more abundant than *subulatus.*

Pipistrellus subflavus is much smaller than the other two. It differs from them in color, in the number of the teeth, the form of the skull and other structural details. In habits, it is less active, both in nature and in captivity. For this reason it is not well suited for experiments.

The Physical Environment.

All the more detailed observations on bats in a state of nature were made in the caves near Mitchell. Since the conditions there are fairly typical of the natural environment of the animals elsewhere, a somewhat detailed description will be given. The accompanying diagram (Fig. 1) will serve to illustrate the relations of these caverns and openings but not their proportions. The arrows indicate the direction of the stream.

Five caves open on this tract of land, or rather there is a

single chain of subterranean passages with five openings. These passages are merely a single underground waterway with a good sized brook covering the floor in most places. At two points the roof has fallen in leaving sections of the stream bed exposed.

FIG. 1. *1*, Entrance to Shawnee Cave; *2*, lower chamber; *3*, blind passage; *4*, large chamber; *5*, entrance to Lower Twin Cave; *6*, entrance to Upper Twin Cave; *7*, entrance to Lower Spring Cave; *8*, entrance to Upper Spring Cave. Arrows show direction of stream.

These openings have been named as separate caves. The size varies considerably, but the average height is, perhaps, six feet and the width ten. In some places it becomes much smaller and the entire passage is filled with water after a heavy rainfall. In other places the size is much greater, in the large chamber (Fig. 1, *4*) the distance from water level to the top of the chamber is about 40 feet and the width at the widest point about 100 feet. There are numerous lateral passages varying in width from a few inches to several feet. A second large chamber is situated near the extreme lower end of the cave (Fig. 1, *2*).

Temperature records kept for a period of two years, in the large chamber at *4*, show an extreme variation from about 51° F. in January, to 57° in September. The air at this point always contains moisture nearly to the point of saturation. Barometic pressure here varies approximately with the surface pressure although the changes take place more slowly. The physical environment of the cave-dwelling bats during their periods of inactivity, is, therefore, nearly constant for all seasons.

Not less than five hundred bats, representing five species, spent the winter of 1906–7 in these caves. Probably the number was much larger, as only those actually seen were counted and some creep away into the smaller fissures where they cannot be found. These bats come out of the cave to secure food only in twilight or darkness in mild weather.

Since the temperature is relatively constant in the cave throughout the year and there is always total absence of light, the problem which first presented itself was to determine how the

animals happen to come out at the right time. For this purpose daily observations were made on the number, location and movements of bats in the large room (Fig. 1, *2*) near the Shawnee Cave entrance and also near the entrance of the Twin Cave (Fig. 1, *5*), throughout the year, excepting at several times when the cave stream was too high to permit access to these places. The large chamber half way between the Shawnee and Twin Cave entrances was visited weekly during most of the year.

Since this work was begun I have visited one or more times about fifteen other caves, ranging in size from unnamed sinkholes to caverns as large as Marengo and Wyandotte in Indiana and Horse and Mammoth Caves in Kentucky. All of them were inhabited by bats, and in all the approximate number and distribution of these animals have been noted, together with such observations on their habits as it was possible to make. Live bats have also been under observation in the laboratory from time to time.

Bats have resting places but no homes. They never construct any sort of a nest or den nor do they habitually return to a fixed spot at regular intervals, although individuals may have a tendency to resort frequently to the same place. Stone and Cram ('02) state that they appear to hang themselves up wherever daylight finds them. These authors give data which indicate that there may be a periodic return to the same spot at short but irregular intervals.

Of the species found in eastern North Ameria some are habitually cave dwellers and some tree dwellers. The habits of the two groups overlap, however, and at least two of the tree-inhabiting species, *Lasiurus cinereus* and *L. borealis* are known to have entered caves in the past.

I have not been able to obtain a reliable record of either of these species living in the caves of the Mississippi valley at the present time. In the large room (Fig. 1, *4*) of the Shawnee Cave more than two hundred skulls of *L. borealis* and two of *L. cinereus* were found scattered among the rocks on the floor of the chamber. Careful searching in the same and other places failed to discover the remains of more than twenty-five individuals of the three species now most abundant there. The skulls, accompanied

by other bones, were scattered among the rocks in a manner indicating that the animals had probably died where they hung suspended from the roof of the cave and that they had not reached the place by accident nor been killed all at one time by a single catastrophe. The age of the remains is difficult to determine. The cave itself is of comparatively recent origin and the bone deposit is evidently much more recent. However, some of the bones must have been there for a considerable period, since they were covered with a deposit of calcium carbonate more than a millimeter in thickness. The remains may indicate that the red bat is a decadent species, represented by fewer individuals at present than in the past, or they may indicate that it has abandoned the cave-dwelling habit in recent times.

During the summer all of the cave-inhabiting species resort to other places, finding temporary homes in attics, deserted buildings, hollow trees and dark nooks in the forest. Merriam ('87) and Miller ('97) have shown that some of the tree-inhabiting species migrate, and there is evidence that *Myotis lucifugus* does also. Just after most of the bats of this species left the Shawnee Cave, about the end of April, 1907, there was a period during which very few were seen flying about in the evening. A few weeks later they were again seen in abundance. It seems probable that the animals which wintered at this place migrated farther north and that the summer residents had passed the winter elsewhere. Howell ('08), describing the diurnal migration of bats near Washington, D. C., states that some of those observed were small and apparently belonged to the genera *Myotis* or *Pipistrellus*. He further states that more than a hundred bats were seen between 9 and 10 a. m. on September 28, 1907. All were flying with the wind in a southwesterly direction, at a height of from 150 to 400 feet. Their manner of flight was unusually steady and consisted chiefly of a sailing or drifting motion with only occasional zigzag movements.

The number and relative abundance of the different species vary without any relation to the size or physical condition of the cave. Mammoth Cave was visited in November, 1907. In a hasty examination of a part of Little Bat Avenue, about 1,000 bats were seen. These were apparently all *M. lucifugus*. The guides

inform me that they are never seen in the inner parts of the cave — probably not more than a mile from the entrance.

Marengo and Wyandotte Caves were visited in July, 1907. Bats never occur in large numbers in the former, perhaps because a building has been erected over the entrance. On the occasion of my visit two were seen, one of them flying, in Mammoth Hall, the other clinging to the wall in the Pillared Palace. In Wyandotte Cave the bats congregate in enormous numbers during the winter. At the time of my visit in summer only a few were seen. Blatchley ('96) states that they reach the innermost recesses of this cave in winter, but gives no localities at which they were seen beyond Crawfish Spring, about two miles from the entrance. The same authority states that he took 401 bats, by actual count, from a space one by one and seven tenths feet square, on a low ceiling in Saltpeter Cave, Crawford County, Indiana.

In the caves of the Donaldson Farm they have been found throughout all of the explored portions, which, in the Upper Spring Cave, extend more than a mile from any known opening. The smaller caves about Bloomington have been visited at intervals throughout the year. In Mayfield's Cave, four and one half miles northwest of Bloomington, the relative abundance of the two most common species is reversed. Banta ('07) states that *P. subflavus* is fairly abundant while *M. lucifugus* was seen only three or four times during three years' observation of the cave. I visited the place January 11, 1907, and December 21 of the same year, and confirmed his observations, finding 17 of the first species and 2 of the second on my first visit and 22 and 3 respectively at the second visit.

P. subflavus was more abundant also in Strong's Cave one mile from Mayfield's, during the winter of 1907–8. In Truitt's Cave, 2¾ miles from Mayfield's, and considerably larger, there were 40 *P. subflavus* and 51 *M. lucifugus* on November 29, 1907. In Coon Cave, 2 miles from Truitt's and 4½ from Mayfield's, there were about 500 bats on March 29, 1908, not more than 50 of which were *P. subflavus*. Two *M. subulatus* were seen and a few others may have been overlooked; the others were *M. lucifugus*. Eller's Cave, visited on the same day as Coon, was inhabited by about 100 bats. Approximately nine tenths of them were *M. lucifugus* and the remainder *P. subflavus*.

Both of these common species have been seen in some very small caves near Mitchell. In one of these, at least, they seem to have wintered, as several were found there on March 26, and a single *Pipistrellus* was seen under a ledge of rock just outside the entrance in February. This cave is merely an irregularly spiral sink-hole going down to a depth of forty feet but without any large lateral passages. All parts of it receive daylight on bright days and the temperature certainly falls quite low in cold weather.

The other caves mentioned vary in size from the two largest known caverns in North America to small caves with not more than half a mile of passages that are large enough to be explored. The entrance to some of them is a vertical shaft, to others it is a horizontal passage going into the side of a hill.

The conditions prevailing within a cave do not determine a bat's choice of a resting place after it has entered. In Coon Cave, as well as several others that I have visited, there is running water at one point and the air here is usually saturated with moisture. In the upper part of the cave, some distance from the entrance, the atmosphere is always dry and the floor and walls dusty. When I visited this cave, bats were about equally abundant in the dry and in the wet parts. In the latter places the moisture had condensed on the animals and drops of water hung from their fur. The arrangement of hairs is such that this moisture does not penetrate to the skin unless the animal is rubbed in moving about.

Usually the animals go far enough into the cave to be in total darkness and a nearly constant temperature, although as mentioned later (p. 163), they sometimes remain for several weeks where they are reached by both light and cold. Blatchley states that "bats choose as a resting place that part of the roof where small portions have begun to flake, giving a certain degree of roughness, or small crevices, to which they can cling. They cannot attach their claws to a smooth surface, hence from large portions of the roof of a room they may be entirely absent." This statement is partly erroneous, for although they cannot attach their claws to a polished surface, the limestone walls and roof of a cave are ordinarily rough enough to furnish adequate support.

I have frequently found colonies clinging to the roof in places where there were no large prominences and no crevices, and I have seen flying bats secure a foothold in such places in the cave, and also to smooth, but unplaned, lumber in a house. The top and side walls are preferred equally by the different species of *Myotis*, but *Pipistrellus* is generally found on the side walls of the higher chambers. The claws of both feet are hooked about prominences on the stone and when the animal is resting on a vertical wall, the wrist and the nails of the thumbs also rest against the wall and form some support. However, the feet alone are strong enough to support the animal for weeks at a time and even to support several others of its kind when they cling to it.

The social habit is strongly developed in *M. lucifugus*. The large colonies seen by Blatchley in Wyandotte and Saltpeter Caves were almost certainly of this species, although he calls them *M. subulatus*. I have never seen them hanging in clusters as large as these but have frequently seen bunches of fifty or more. The guides at the former of these caves tell me that bats gather there in winter in clusters comparable only to a swarm of bees, and probably equalling such a swarm in number of individuals. *Myotis subulatus* and *M. velifer* are not abundant in this region but are generally found associated with groups of their abundant congener.

Corynorhinus macrotis has only been seen in dim light near the entrances, and there it was found clinging to the side walls with its long ears folded down along the sides of the neck. *Eptesicus fuscus* has not been seen far within the caves nor is it abundant. The largest number I have found in one place is six, taken near the entrance of Mayfield's cave on December 21.

Pipistrellus subflavus is solitary in habit. Occasionally two are found side by side, though I have never seen them clinging to each other except in mating. However, they do not avoid the vicinity of others of their own kind nor other species. This species seems to prefer the side walls of the higher passages. I have never seen it suspended from the roof except where there was a crevice or prominent ledge.

Enemies.

Very few enemies molest bats in their roosting places in the caves. In Eller's Cave I saw evidence that raccoons had been preying on them. It is said that cats have learned to catch the flying bats in Wyandotte Cave. No doubt other carnivora sometimes kill them, but on the whole they are practically free from molestation in the caves. While living in trees they are doubtless preyed upon more frequently. In two instances I have known them to be driven from their roost by birds, once by a robin and once by a blue jay. The barn and great horned owls and the sparrow-hawk have been known to eat them in rare instances.

Their enemies are so few, however, that they have no sense of fear comparable to that of other small mammals. A sharp noise will sometimes startle them into activity. If wide awake they may fly before they can be picked up by hand or net, or if caught they often struggle to get free. But there is never any attempt to "lay low" or to flee from approaching danger. When kept in cages they do not pay the slightest attention to the presence of man, nor try to escape his hand if he attempts to pick them up, even when first brought into captivity. The absence of fear has a marked effect upon the habits and mental life of the animals. Only in such species as are without natural enemies is it possible that there can be such long periods of inactivity or such a deep lethargy in normal sleep.

Periods of Activity and Rest.

Bats are usually active only in the twilight and darkness during warm or moderate weather. The earliest date at which I have seen them flying at a distance from their dwelling place was March 3, 1906, at Washington, D. C. The evening was balmy but there was snow on the ground in places. The latest date I have seen them was November 8, 1906. The evening was warm but had been preceded by some hard frosts. I have seen them come to the mouth of a cave in midwinter and turn back when they felt the cold air. On warm winter nights they no doubt prolong these excursions.

Since they live a part of the time in the caves where there is a total absence of light and where the temperature varies only a few degrees throughout the year, the question has been asked, how do they know when to come out (Blatchley, '96)? The answer is, they try conditions and only come out under favorable circumstances. So far as I know, careful observations bearing on this point have not hitherto been made and it seems worth while to record my own in some detail.

In the first place, hibernation among bats is not strictly comparable to the same process among the lower vertebrates, since it is not one unbroken period of torpor more or less dependent on temperature (Oldham, '05 ; Rollinat and Trouessart, '96). The vital functions of a frog may be practically suspended during a long period, the lungs and digestive organs almost ceasing to function for the entire winter. In bats the activity of the vital organs decreases, though only to a limited degree. The rate of respiration is difficult to count accurately because the body movement is slight. I have counted the respiration of a dormant bat in the cave at several times during the winter and found the rate to be about 60. At other times I have seen the animals apparently cease to breathe for periods as long as four and a half minutes, and then after one or two convulsive respirations, the frequency would suddenly go up to as high as 82 per minute. I am not certain that breathing actually ceased during the quiescent period but there was no visible body movement. In any case, the same conditions are found during profound diurnal sleep at all seasons of the year.

The animals do not obtain food during the winter but the stored fat is used up and wastes are excreted from the body. Oldham ('05) found fecal matter in the intestine of the lesser horseshoe bat (*Rhinolophus hipposideros*) during the winter and regarded it as proof that the animals had been eating recently. However, fecal matter is, in part, derived from wastes excreted through the wall of the lower part of intestine and is not dependent upon food. The presence of feces is, therefore, no indication that an animal has recently eaten. Lusk[1] states that a fast-

[1] "Science of Nutrition," Graham Lusk, p. 46.

ing dog weighing 30 kilograms excreted 1.88 grams of fecal matter per day. In the large number of bats which I have dissected in winter, the stomach and upper part of the intestine was always empty, although feces were present in the rectum. I am convinced that the cave bats of southern Indiana seldom or never eat during the winter, the stored fat being sufficient to sustain life.

The degree of lethargy bears a close relation to the quantity of superficial fat stored up by the animal and it is not related to the temperature, either without or within the cave, nor to season. In fact, the period of least activity is in the autumn and early winter, before severe weather has begun. The bats are often quite active in the cave during the cold weather of late winter. Between August 8 and September 5, 1907, I took a number of bats, *Pipistrellus subflavus*, *Myotis lucifugus*, and *M. subulatus* from the caves to use in experiments. Some of these were very fat while others were comparatively poor. On September 5 I used two males of *P. subflavus*. The first one was quite poor and flew readily when released in the room after being carried for a short distance in a small box. The second bat had to be prodded and tossed about before it could be awakened from its lethargy sufficiently to fly. Even then its movements were more sluggish and it struck obstacles oftener and also had to be frequently disturbed in order to keep it in motion. I have never seen a bat more difficult to arouse at any time during the winter nor one more torpid when once induced to fly. An examination showed that this animal was exceedingly fat. This is not an isolated case but merely illustrates what has been found several times in this and other species.

When in a state of lethargy, a bat cannot be quickly aroused. Neither noise nor light appears to be a sufficient stimulus to awaken it. Heat will arouse it more quickly than any other stimulus and it will immediately draw away from the heat of a candle. Mechanical stimuli are also effective and bats are sometimes aroused from torpor by being carried for a distance. Merzbacher ('03) found that the reactions of hibernating bats are similar to those in which the cerebral hemispheres have been destroyed. The clinging reflex is very evident, even in the most torpid animals. In the torpid state the body temperature falls

to such an extent that the limbs and membranes feel cold to the touch. As the animals are aroused, the breathing becomes stronger, the temperature rises, the eyes open and often there are convulsive movements of the limbs. The animal may also begin to chatter and to creep slowly. If laid on its back it slowly rights itself. However, it is some time before it gains full control over its muscles. If dropped, the wings spread reflexly, but the animal cannot at once fly. There are intermediate stages of lethargy in which the torpor is less extreme and the animal very quickly gains power over its body, but the extreme conditions described above have been observed in every month of the year except May, June and July, during which months but few bats have been under observation.

Observations on periodic movements of bats were made chiefly at two points in the caves at Mitchell, in the large chamber (Fig. 1, 2) at the right of Shawnee Cave, and at a point about 100 feet within the Twin Cave entrance (5). Both points are so near the entrance that the temperature varies considerably. On bright days a diffuse light reaches both points for an hour or more when the sun's rays fall directly into the mouth of the cave. Daily observations were recorded from January to April, 1907, with the exception of several times when high water prevented entering the cave.

On January 2, with a maximum temperature of 50° F., there were 75 bats at the place of observation in the Twin Cave. A period of low temperature followed, and high water prevented further observations until January 25, when the number had decreased to 9. The number now increased gradually until February 7, when there were 51, although the temperature remained low. With the average temperature slightly rising, the number of bats diminished during the next two weeks to 42 on the twenty-first of the same month. During the next four weeks the number of bats again increased until on March 20 there were 101. The temperature had been rising gradually and with some fluctuations, and the average daily temperature on March 20 was about 45°; a further rise to 70° on March 27 followed. With these higher temperatures the number of bats on the twenty-eighth of that month was only 18. Unseasonably

cold weather during April was accompanied by an increase in bats which reached a maximum of 153 on April 20. Observations carried on during the same period at 2 (Fig. 1), which is another part of the same cavern, showed a variation in the number of bats near the entrance which almost exactly paralleled that in Twin Cave, thus showing that the movements had some common cause and were not wholly accidental.

Apparently the movements have a definite relation to season and temperature. The bats come to the mouth of the cave at intervals throughout the winter, but these intervals are longer in the early winter when the animals are fat and well nourished. The unusually warm weather prevailing early in January may have acted as a stimulus for them to remain near the entrance; no doubt some individuals left the cave at night in search of food. Cold weather followed and the cold, entering the cave, drove the animals back to the warmer parts. However the hunger stimulus was becoming stronger and the bats came to the entrance more frequently and tended to remain there. The maximum number was reached with a moderate temperature, and when the weather became quite warm the animals left the cave and did not all return but found temporary homes in trees and buildings. Cold weather in April brought them back to the cave again, but most of them remained near the entrance. When the weather again became warm at the end of April, they left the cave for the summer.

Other observations were made on the movements of individual bats at different times. The location and orientation of different individuals were carefully marked and the place was visited weekly. Out of 18 bats observed between November 19 and December 3, 14 had moved within one week, and none remained in the same place during two weeks. Later in the winter one bat remained in the same spot near the entrance from February 4 to 27. Light reached the spot throughout the day and the temperature remained near freezing point for several days at a time. However, this was an exceptional case, as not many bats remained in one location for more than four or five days during the latter part of the winter. The small bat, *P. subflavus*, is less active. Its average period of staying in one place is about two weeks, and one was noted in the same spot for 44 days.

Data on the daily movements of bats are still very meager. Moffat ('05), who has observed Irish bats, states that among the Irish bats, Daubenton's, the pipistrelle, and the long-eared fly all night. The Noctule flies in the evening twilight, and the hairy-armed flies for about an hour shortly after sunset, then retires to its roost and again comes out shortly before sunrise. Six pipistrelles, living solitary, were found to have similar, and very regular habits, leaving the holes in hollow trees from ten to thirty minutes after sunset, and returning from forty to eighteen minutes before sunrise. The observations, which were made in August, showed that there was no difference for warm nights and raw, cool nights. Morao ('63) states that the bats in an immense colony of *Myotis lucifugus* in the attic of a house near Charlestown, Maryland, were accustomed to leave their roost twice in one night. His somewhat poetic statement that they left at "the call of the whip-poor-will" cannot be considered accurate, for in such a large colony, individuals could not be noted and any general disturbance of the colony might be mistaken for a renewal of the activity of the individuals.

The same difficulty exists with regard to determining the daily activity of bats in a cave. Their dwelling place is so large, and the possible exits usually more than one, so that their movements cannot be watched. It is possible to go into the cave and examine individuals, but there is the danger of disturbing them and causing them to leave sooner than they would if unmolested.

I have seen bats flying in the cave at all hours of the day and night, and have also found them at rest there at all hours. Evidently they may awaken from sleep at any time and fly to the mouth of the cave. If the temperature and light are favorable they go out and search for food. If it is cold or if the light is too strong, they go back. If they are fat and well nourished they settle down to another more or less prolonged period of lethargy. But if the hunger is strong they are apt to remain awake and active, or only go to sleep for a short time.

The bats in the clusters seen in spring or late winter were usually awake and chattering. In the fall and early winter bats are generally isolated and torpid. Those that are active are very apt to reach the mouth of the cave as soon as conditions out of

doors are favorable for their activities. The presence of bats in the cave at night, when others are out searching for food, shows that there is no definite time at which they all leave their roosts. This fact has also been observed with regard to the European pipistrelle (*Pipistrellus pipistrellus*) by Whitaker ('07), who says that only part of a large colony left their roost under a roof on a certain night. If a large percentage of the animals does become active at about the same time, it must be remembered that many of them pass the summer in places where daylight reaches them and the absence of light as night falls may be a direct stimulus to activity. Falling temperature at sunset may also stimulate the animals to activity when they are not in the caves.

On rare occasions the hunger stimulus may be so strong as to overcome the natural repugnance to light, and the animals come out to search for food in daylight. I have witnessed this but twice. In late autumn a *Pipistrellus* was seen circling high above the trees, and at another time, May 9, 1907, a *Myotis lucifugus* was seen feeding in the bright noonday sun near the mouth of Shawnee Cave.

On the average, a bat certainly does not fly more than six hours out of twenty-four, and that for not more than eight months of the year. At least five sixths of its life is spent hanging head downward in the dark.

From the foregoing facts we may assume that a bat's life is made up of a series of alternating periods of torpor and activity. The relative and absolute length of these periods depends on the state of bodily nutrition.[1] When the body is well nourished and the quantity of reserve fat large, the periods of lethargy are long and the time of activity short. As the stored fat is used up the periods of lethargy become shorter and active states longer and more frequent. During the season of greatest activity, from May to July inclusive, the times may correspond to daylight and darkness, and the condition of the animals to ordinary sleep and activity. However, the longer periods have no direct relation to

[1] The physiology of hibernating bats has been studied by Rulot ('02) and Merzbacher ('03). According to the former, glycogen and albumen are consumed during hibernation, especially toward the end of the period. It is evident that the hibernating state in the bats studied by these authors is more profound than it is in the bats which I obtained in the caves.

season or temperature nor are any of the periods dependent upon the physical environment.

Feeding Habits and the Perception of Food.

The feeding habits of bats are by no means easy to study. They habitually secure their food while flying, and then only during twilight or darkness when it is impossible to distinguish their movements accurately. In a state of nature their food consists largely, perhaps wholly, of insects. The single time that I have seen a bat feeding in daylight near enough to distinguish its prey, it was catching small ephermerids and diptera. At dusk they can sometimes be seen pursuing larger insects, apparently beetles. The food is so thoroughly masticated that examination of stomach contents furnishes no definite clue to the identity of the things eaten. Neither does the food that an animal will eat in captivity afford an index to its natural food. Meal worms seem to be the favorite article of diet of captive bats. Fresh meat is eaten readily. They will also eat a small worm (*Tubifex*) which lives only in mud and certainly is never eaten by the animals in nature and has not been by their ancestors since the flying habit was acquired.

Dobson ('78) states that a fruit bat (*Cynopterus*) which he captured in Calcutta consumed a banana twice its own weight in three hours. Whitaker[1] states that the hairy armed bat, *Ptergystes leisleri*, eats about five dozen meal worms a day, and that a female noctule, *P. noctula*[2] after several days fasting, during which she gave birth to young, consumed eight dozen meal worms in one evening. None of the bats which I have had in captivity have been voracious eaters. Captive bats will learn to eat meal worms greedily when they are offered to the animal with the fingers or a pair of forceps. Only on one or two occasions have I ever seen a bat pick up food from the floor. When a meal worm is taken between a pair of forceps and held before a bat, the animal will snap at it eagerly, especially if the worm is wriggling. However, its efforts are not well directed and it is as apt to get the forceps in its mouth, or to miss the objects completely, as it is to seize the worm.

When food is accidently dropped the bat does not make any

[1] '07. [2] '05.

attempt to recover it and does not even turn its head to look for the lost morsel; generations of flying ancestors have not found it advantageous to try to recover an object dropped while on the wing.

Bats are not wholly dependent on a single sense for distinguishing their food. Smell, on which many other mammals are chiefly dependent, here is of subordinate importance. The reason is to be found in the way in which food is secured. Any creature walking on a solid surface and having a characteristic odor, can be located, or can be traced some time later, by an animal with a keen sense of smell. But flying insects, which form the chief food of bats, do not leave a permanent odor in their path nor can their presence be definitely localized because the odors are diffused too rapidly and unevenly by shifting currents of air. I have held meat, meal worms and insects near a hungry bat and it did not seem to notice their presence until some sense besides smell was stimulated. However, fresh meat fastened to the side of the cage was found and eaten after a time. On one occasion a bat that was running across the floor of a cage perceived a piece of meal worm it was passing and picked it up. This was, however, the only instance of the sort I have observed in handling and feeding a large number of bats. The same animal and others seemed quite unable to find meal worms or insects lying quietly on the bottom of the cage. Occasionally they found meat placed in a small dish. This happened more often with *Eptesicus* and *Pipistrellus* than with either species of *Myotis*, though the latter found the meat more readily when it was fastened to the side of the cage so that the animals climbed about over it.

It must not be inferred from the above statements that the sense of smell is lacking, or even rudimentary. All bats have a strong odor, the purpose of which is probably to attract others of their kind. This may be taken as an indication that smell is well developed, for otherwise the odor would be useless. The action of the animal mentioned above in stopping to pick up the meal worm, and the ease with which others learned to find and eat such unfamiliar food as meat, also indicate that the sense of smell is not lacking. The fact that they do not usually notice food when it could be perceived by this sense alone, indicates only that they are not accustomed to find it in that way.

After extended observations on the subject, I am still unable to form any definite conclusions with regard to the importance of sight to these animals. That they can see light and darkness and moving objects is unquestionable. That the sense of sight is not highly developed is equally certain. The behavior of some of the animals appears to indicate that at times they depend on this sense to a considerable degree, both in securing food and in avoiding objects.

On bright nights, and in twilight, a dark, moving object can be readily seen against the skyline. Under such circumstances sight would be of use to bats in helping them to find the general location of food. Whether it really guides them at such times is a point not yet determined.

Bats are extremely sensitive to vibrations of high frequency. A sharp whistle, sucking noises with the lips, tearing a sheet of paper and drawing the finger nail across a piece of thin board or rough cardboard cause them to start violently, but low pitched, rumbling noises have no apparent effect.

Flying insects usually produce a high-pitched hum. While it would be of advantage to a bat to perceive these sounds, the evidence that they are actually guided to their prey by hearing them is inconclusive. It is not possible in observations on feeding to distinguish between response to hearing and to tactile stimulation, by the vibrations. The voice of different species of bats varies but it is always high pitched. Alcock ('99) states that the voice of the hairy-armed bat has about 17,000 vibrations per second. The pitch has not been determined for the voice of the American species.

It is evident, however, that it is the *motion* of the insects that lead to their perception by bats. Whitaker ('06) states that a noctule which he observed caught a pebble tossed into the air. In this country boys often gather under the electric lights or at the edge of a wood where bats are abundant in the evening, and knock them down with a fishing pole waved rapidly in the air. In both cases the bats are attracted by the moving object and probably by hearing. The tactile sense, located in the vibrissæ and lips, is certainly very delicate and doubtless aids the animal to definitely locate its food. In feeding meal worms to bats I

have found that the animals do not, as a rule, pay any attention to worms held near them so long as they are quiet. But when the worms begin to wriggle the bats at once become excited and begin to snap at them. This happens when they are not touched by the worms and when the latter are out of the range of vision. It seems improbable that touch is the sense here aroused. The food must have been perceived by the tactile organs being stimulated by air currents set in motion by the moving worms.

It is said that bats use the interfemoral membrane, which the flying animal carries curved downward and forward under the body, as a sort of scoop in which insects are caught. It is possible that food is thus secured at times, but it is more often seized in the mouth. However, the membrane is used as a pouch into which the bat thrusts its head when it has an insecure hold on an insect. The membrane thus serves as a pouch to prevent dropping the food and also serves as an object against which the struggling prey can be pressed while a firmer hold is being secured.

Observers (Whitaker, '06; Grabham, '99), who have studied the habits of various European species of bats agree that they drink while on the wing, flying over a body of water and dipping down to its surface to drink. I have observed the same habit in *M. lucifugus;* it probably alights to drink also. When in captivity this species learns readily to come to a small cup of water placed on the floor of its cage. The animal gets up on the edge of the dish, resting on its wings and body and bracing with its feet. Often it dips a part of the forearm and wing into the water. The lower jaw and tongue are thrust in, the mouth is filled with water and, generally, but not always, the head is raised to its normal position and the water is taken down in a succession of rapid swallows. On the whole, the method of drinking resembles that of a young chick, except that the head is not lifted so high.

If the conclusions given above as to the manner of perceiving food are correct, it is obvious that water must be perceived in some other way, since it is obtained where it is relatively stationary and noiseless. On two occasions I have seen bats in the laboratory apparently attempt to drink while flying. On the first occasion there was an aquarium of running water in the room. The bat

flew near enough to this to feel the splashing water and then turned and flew repeatedly across the room, keeping near the floor and frequently giving the floor an audible bump with its opened lower jaw. The other time there was no running water in the room which could have set off the impulse, but there was standing water which it may or may not have approached. On both occasions there was a good light, either artificial or daylight, and the floor was of a dull, yellowish brown color which might look to an animal flying over it like water of a pond on a starlit night.

The evidence at hand is not sufficient to prove the point, but it seems probable that sight may be the sense by which water is usually distinguished, but that moisture-laden air, rising from a body of water to a bat flying above it, also helps the animal to locate water.

I am unable to say whether bats ever drink in the caves. In most places there is so much moisture that they probably do not become thirsty. There is no evidence to show that they ever eat in the caves. Some insects could be obtained there but the quantity would be inconsequential as compared with the number of bats to eat them. The lack of sufficient food is doubtless the only reason that they have never become true cave dwellers.

Locomotion.

Bats are more helpless on their feet than most birds. This is in part due to the mechanical impediment of the flying membrane, and in part to the skeletal modification outlined in the section on morphological characters. As a result of these changes in form the animals cannot support themselves on their hind limbs alone, as do birds and man, nor can they rest upon the terminal part of the fore limbs. When walking upon a horizontal surface a bat rests upon the sole and claws of the hind foot and upon the carpus and thumb of the fore limb. The phalanges are usually folded backward along the fore arm, as when at rest, though the wing is sometimes slightly expanded. The tail and interfemoral membrane are curved forward under the body and both the tail and the wing may touch the floor at times. The body is elevated so that it clears the floor. The limbs are moved as in other

mammals, the right hind limb being lifted with the left fore limb, and vice versa. The steps are necessarily very short because the membranes prevent long steps, although they are sometimes quiet rapid. This rapid movement across the floor has been very well described as "scurrying." It is never kept up for a long distance. The animals apparently become tired in a run of a few yards.

Flying is the usual mode of locomotion for bats and they have the capacity for flight developed to a high degree. We have no definite information as to the speed of a flying bat, the duration of its periods of flight, nor the distance that it will travel from either its birthplace or its temporary dwelling. Some of the animals that I have had in captivity seemed to tire very quickly and could not be easily induced to take to flight when they had once settled down. Attempts to estimate their speed can be scarcely more than a guess because their erratic, wavering flight is much more difficult to measure than that of a bird, and because of their nocturnal habits. *Myotis lucifugus* probably flies at a rate of about ten to twelve miles an hour. *E. fuscus* flies faster and *P. subflavus* not so fast. The flight of the last named species is weak and wavering and resembles that of a butterfly. *E. fuscus* has a relatively rapid, strong and steady flight, while *Myotis lucifugus* and *M. subulatus* are, in a way, intermediate between the two.

The quick turns and evolutions which bats make as they fly about in the twilight are for the purpose of catching flying insects. However, their manner of flight is essentially the same when they are not feeding. It may be that this erratic flight has some relation to the kind of place in which these animals are accustomed to live. In the earlier stages of the evolution of flight, bats must have lived in trees and their movements must have consisted of short leaps or flights among the branches, where skill in avoiding the limbs and in clinging to them was of more consequence than steady or prolonged flight. As the power of flight became better perfected the animals would still secure their food largely among the trees, but would remain on the wing longer and would dart here and there among the branches snatching food as they went. Hence the importance of being able to readily perceive and avoid small objects. The cave-dwelling habit would tend to further develop

these peculiarities, since the angles and projecting ledges of the caves would prevent a straight and continuous flight. Catching insects on the wing would make agility count for more than endurance and steadiness, and hence the characteristic mode of flight has been preserved.

The migrations previously mentioned (p. 155) would seem to indicate that individuals may travel five or six hundred miles twice a year. The steady flight noticed by Howell ('08) in diurnal migration may be taken as an indication that these animals make long, continuous flights and have considerable endurance. In this characteristic we find another analogy to birds.

A flying bat can change its course or check its momentum very quickly. When it does not perceive a solid object that it is approaching, it sometimes strikes its head while going full tilt and falls down. Usually, however, an object is perceived before actual contact takes place, and in that case the animal is always able to check its flight and alight on the obstacle if it is too near to turn aside and avoid it.

The quick turns and dodges seem to be made by changing the angle of the wings either antero-posteriorly or dorso-ventrally. The interfemoral membrane and tail may act as a rudder, but a bat from which they had been removed flew as well as before the operation.

A flying bat can alight on a vertical wall in several different positions. Oldham ('05) states that the British Vespertilionidæ alight on vertical surfaces with the head upward and reverse quickly after obtaining a foothold, while the lesser horseshoe bat (family Rhinolophidæ) reverses in the air and alights head downward. Both of our common species of *Myotis*, and I think all of our other cave-inhabiting bats, can reverse in the air and alight head downward although they do not always do so. When flying against a window screen or some other object, not perceived until it is almost touched, they alight head up, striking with the anterior end of the body first and letting the posterior end settle down.

The reversal consists in a sidewise dip with wing and head, the hind limbs being brought forward and thrown upward at the

same time so that the one wing is directly above the other; the sidewise motion then continues far enough to bring the head under the tail and the claws of the feet grasp the surface. If the wall is too smooth to furnish a foothold the bat is in position for immediate flight. Sometimes the position is only partially reversed and the animal alights sidewise. In this case the thumbs support most of the weight.

A flying bat can secure a foothold upon a horizontal surface beneath which it is flying as easily as on a vertical wall. To secure a foothold the bat throws its head downward and its feet upward and forward till they touch the roof and the claws grasp the supporting object. The quickness with which the momentum of flight is checked is one of the nicest adaptations of a bat's life. Only a slight roughness is necessary for the sharp curved claws to secure a firm hold. I have seen a flying bat clasp and hold a vertical number 16 wire that it accidentally struck. The fore arms were placed behind the wire which was pressed against the back as a man might hold a cane thrown across his shoulders. A bat in flight can catch a rafter or similar object by a single thumb, or by the claws of one foot. Metal, glass, polished wood or stone are not rough enough to furnish support, but unplaned boards, and rough limestone, furnish adequate foothold.

When a bat launches into flight from a perch on the roof or side wall, it always drops downward, spreading the wings as it drops. It can launch into flight from the floor or other flat surface, but it cannot rise vertically in the air from a resting position. A bat which fell into an empty aquarium, 16 inches in diameter, and the same depth, was unable either to climb its smooth sides or to fly out of it. When caught in a dip net they are unable to fly out of it, but must climb the sides and fly from the rim to escape, a fact which makes it easier to capture them.

Breeding Habits.

The reproduction of some of the European bats belonging to the families Rhinolophidæ and Vespertilionidæ has been studied by several zoölogists. Benecke ('79), Eimer ('79), Van Beneden and Rollinat and Trouessart ('96), all state that copulation takes place in late summer or autumn. The spermatozoa fill the lu-

men of the uterus and remain alive but inactive throughout the winter. Ovulation and fertilization take place at the return to active life in the spring and development begins at once and continues without a resting stage. Duval ('95) states that bats copulate a second time immediately after hibernation, but Rollinat and Trouessart ('96, p. 220) consider his observations to be erroneous.

The reproduction of American bats has not been studied in detail. I found *Myotis lucifugus* copulating in Shawnee Cave on October 27, 1906, and at two unrecorded dates shortly afterward. In Truitt's cave I saw a pair copulating on October 19, 1907. Two pairs of *Pipistrellus* were apparently copulating in the same cave on November 29, but they were too high to be reached and I could not be certain. A pair of *Myotis subulatus* appeared to be copulating early in April, 1907, but they also were in a position where they could not be obtained or be carefully watched. If mating actually took place at this time it may have been the post-hibernal mating mentioned by Duval, as it is extremely improbable that this species would copulate at a very different season from its near relative.

In sexual congress the female clings to a vertical wall or ledge. The male attaches himself to the posterior part of the body of his mate, and clings to her fur and membranes with his claws, but also rests in part on the interfemoral membrane and body. The posterior portion of his body is flexed forward, pushing aside the interfemoral membrane of the female, so that contact between the genital organs can take place. Coues and Yarrow ('75) state that the red bat (*Lasiurus borealis*) copulates during flight, but this statement is so at variance with the facts observed for the other species that it cannot be accepted without further confirmation.

The uterine contents of *M. lucifugus* were not examined to determine at what time fertilization takes place. However, embryos were not present in any that have been examined in the caves, including several as late as April 9, and one on April 27. It can be asserted, that in this species, development does not begin until the beginning of the summer activity of the female. After this time the females seldom or never enter the caves and I have

not been able to find them at all during the period of gestation and the rearing of the young, nor have I ever found the young bats of either species of *Myotis* before they had reached adult size.

Pipistrellus likewise leaves the cave for the breeding season, although I have taken a female of this species containing three small (about 2 mm.) embryos in the Twin Cave on June 6.

The males of certain oriental species of bats (*Chiromeles torquatus* and some of the species of *Cynopterus*) have special adaptations for carrying the young. The Standard Natural History (p. 161) generalizes from this fact so far as to say that "it is not doubtful that the male attends to his mate and young with considerable assiduity." The absurdity of this statement in so far as it applies to our common Vespertilionidæ, is apparent from the further statement on the same page that "the sexes do not mingle and come together only at the nuptial season." There is almost certainly no permanent mating but the animals copulate indiscriminately, several males perhaps mating with one female. This is what might be expected in gregarious animals that do not rear their young in a nest or den, but give birth to them at any convenient place and carry them about. Rollinat and Trouessart ('96) believe that this is what happens in the case of *Pipistrellus pipistrellus* and *Vespertilio murinus*.

The females of our species of *Myotis*, and perhaps the other Vespertilionidæ of eastern America, probably seek out isolated places in which they give birth to the young and where they spend most of the time while rearing them. As long as they remain in the cave in the spring there is no complete segregation of the sexes. I have found the two sexes associated in Twin Cave on different dates in April (the latest examination was made on April 25) both in the years 1907 and 1908.

The females leave the caves somewhat earlier than the males. On April 25, 1908, in a search through the outer parts of all the caves on the Cave Farm, I found 23 male *P. subflavus* and 4 females. Twenty-five male *M. lucifugus* were also found, to only 2 females of that species. On May 13, in Truitt's Cave, there were 17 *P. subflavus* and 5 *M. lucifugus*, all of both species being males.

The Behavior of Bats in Captivity.

Bats in captivity, as well as those at liberty, are very erratic and uncertain in their behavior. Some of them are sluggish and cannot be used at all for experimentation. Others are quite active for a time and then suddenly retire to some corner, hang themselves up by the feet, and do not move from their chosen position for hours, or even days, unless they are disturbed. If disturbed, they sometimes open their mouths and chatter angrily, but do not move unless forcibly pushed aside. Others will fly a short distance and then settle down again. Occasionally a repeated disturbance will arouse them to complete activity.

They learn to go and drink from a small dish of water placed in their cage. Some have learned to go to a dish of raw meat and eat. As a rule, however, they do not find food on the floor of the cage, but will eat more readily if meat is placed on the sides, where their head comes in close contact with it as they move about.

Insects are not readily eaten unless presented to them with fingers or forceps. Meal-worms are eaten with much apparent relish, but often, especially in winter, even this food has to be thrust into their mouths so that they will taste it before they learn to eat. I have often turned the meal-worms loose in a cage or small box with bats, but not one has ever been picked up as it was crawling around, although they sometimes crawl over the animal's body and membranes.

The manner of eating, and the time required for the consumption of the same amount of food, varies considerably at different times and with different individuals. They eat slowly as compared with other animals of equal size; from one to five minutes being required for eating a single meal-worm. Some swallow only the juices and soft parts, letting the chitinous shell pass out of the corners of the mouth.

During the winter 1907–8 my captive bats were kept in small glass and wire cages that were placed in a small photographic, dark room. The door was never closed tightly, except temporarily, so the darkness was not complete and ventilation was fairly good. The temperature varied somewhat but never fell below 40° F. nor rose above 65°. The animals spent most of

the time clinging to the sides of the cages near the top, but went down now and then to get water. As long as they were undisturbed they moved about little and remained in good health. Some *M. lucifugus* obtained in Mammoth Cave, November 8 were kept alive until March 26. When taken out and held in the hand or placed in a warm room and touched occasionally, their temperature gradually rose, and in from ten to fifteen minutes they usually began to creep about, and then to fly. For some reason that I have not discovered, the animals never lived long when they were disturbed frequently. From February 10 to March 3 was the longest that I was able to keep a bat in good health when using it daily for experiment. It is possible that the dry atmosphere of the steam-heated rooms is not suitable for them.

A characteristic of bats, liberated in a large room where they can fly about, is the tendency of an individual to alight frequently in the same place.

Another noteworthy tendency is that of exploring every nook and corner of a room. It results in finding any crevices through which it is possible to escape. This tendency must have been of incalculable importance to animals accustomed to spending much of their time in dark retreats, reached only through small and winding passages.

(*To be continued.*)

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SOME HABITS AND SENSORY ADAPTATIONS OF CAVE-INHABITING BATS. II.

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Experimental Studies.

1. *Avoidance of Objects.*

As early as 1794 Spallanzani experimented with bats in which one or more of the senses had been destroyed. I have not had access to the original account of his experiments. According to the account of these experiments given by Godman ('26) and Flower and Lydekker ('91), bats deprived of sight, hearing and

smell, were able to avoid objects in their way and even silken threads stretched so that there was just room for the animals to pass between, and they contracted the wings when the space was too narrow for the expanded wings to pass through. Observations on a large number of captive bats have convinced me that Spallanzani's experiments were, in some way, lacking in scientific accuracy. Not a single individual out of more than sixty belonging to five species that I have experimented with, have shown any approach to this degree of skill in avoiding objects, even with the senses all intact.

The experiments here described were made at the "University Farm" in the spring and summer of 1907, and were checked by additional ones in the laboratory of Indiana University in December of the same year. They were similar in part to those made by Rollinat and Trouessart ('00), with, however, a simpler arrangement of obstacles and with each experiment worked out quantitatively.

The experiments show that bats are able to avoid objects when flying, but that avoidance is not complete. Several senses may be of use in perceiving obstacles, and air currents perhaps guide the animals to some extent. However, destruction of the sense of sight does not seriously impair their ability to perceive objects nor does the loss of the external ears and tragi. The most important senses are located in the internal ear and any disturbance of these organs seriously impairs the animal's ability to perceive and avoid obstacles.

The following method was used: The bats were liberated in an unceiled room approximately fifteen feet wide, eighteen feet long, nine feet from floor to eaves and twelve feet from floor to the apex of the roof. Pieces of black, annealed iron wire about one millimeter in diameter were suspended from the rafters and kept moderately tight by fastening the lower ends to a cross wire five feet from the floor. On an average, there was one wire to each eleven inches of space, but they were spaced unequally, the purpose being to determine whether the bats would try to pass through the more narrow spaces or learn to select the wider ones. During a part of the experiments there was an additional row of seven short wires, alternating with the others and placed twenty inches from

the first row, but they were high in the comb of the roof, the bats seldom passed between them, and as they had no apparent effect upon the experiments they will be counted as though all were in one series.

Wires were used in these experiments in preference to larger objects because a bat will invariably try to perch on any object it strikes and it is sometimes difficult to tell whether the animal intended to perch or whether it did not perceive the object. Wires have an advantage over strings because the slightest touch causes a perceptible sound and it is not always easy to *see* whether an object is touched. Some preliminary experiments were made in April but the method of quantitative study was not decided upon until May 16, when one bat was used. Several more were tried during May and June but at this time few bats were in the cave. The experiments were therefore discontinued until later and most of them were made between August 25 and September 5.

Some of the animals were very fat and inactive during late summer and it was difficult to keep them flying. There are great individual differences and some of the experiments required six times as long as others and some bats had to be discarded for the purpose of these experiments because they would not fly at all or would make short flights in one corner of the room and not attempt to pass between the wires. The tabulated results were obtained from the use of 48 bats belonging to three species. About fifteen additional individuals and two additional species were liberated in the experiment room but are omitted from the tables because the data are incomplete. The total number of observations on avoidance is about 6,000.

The individual differences and the varying degrees of lethargy in the same individual at different times make it essential to determine the normal reactions of each animal before trying experiments under changed conditions. For this reason it was deemed necessary to test each individual in a normal and uninjured condition immediately before the experiment in which one of the senses was impaired.

The bats were captured in the cave and were generally used for the experiment on the same day, although some of them were

not used until the next day. The normal, uninjured animals were liberated in the room and their movements carefully watched. Each time one of them passed between the wires or approached quite near to a wire and appeared to dodge it was called a trial. It was at first intended to allow 100 trials for each bat but it was found that the animals were apt to become tired and refuse to fly before the experiment was concluded and the number of trials was reduced to 50 for each condition. The wings brushing against the wires, even very lightly, produce an audible sound so that it was easy to tell when the animal struck the wires.

Most of the normal bats flew about the room rapidly for a time and then began to stop frequently, alighting on the walls, the underside of the roof or objects in the room. After a period of varying length some of them attempted to settle down and it was difficult to keep them on the wing. On being driven from their perch they would make short flights only, and stop again. For reasons previously stated (p. 167) striking objects other than the wires were disregarded.

Four kinds of mutilation were employed: (1) The eyes were covered with an opaque mixture of lamp black and glue. (2) The external ears and tragi were excised close to the head. (3) The external auditory meatus was stopped with a small quantity of plaster of Paris which was allowed to harden before the bat was liberated. (4) The hairs of the body and membranes were pasted down with thick vaseline.

When the eyes were covered with the mixture of lamp black and glue, the most noticeable effect was to decrease the activity of the animal. Usually a bat so treated alights somewhere and tries to remove the substance from its eyes, using the hind foot, as the thumb and wrist cannot be brought into contact with the head. If the glue is allowed to harden somewhat before the animal is liberated, it is not so easily removed; even then it is necessary to dislodge the bat as soon as it alights or it will finally succeed in removing the hardened glue. In every instance the animals were examined at the conclusion of the experiment to see that the covering was intact.

The 47 bats used in these experiments struck the wires 25 per cent. of the 2,350 trials recorded for the uninjured condition.

Twelve of the forty-seven were blinded in the manner previously described and given fifty trials each. In these 600 trials the percentage of hits was 21.7. However, the percentage of hits for the same twelve in the normal condition was only 23.6 per cent. as compared with 25 per cent. for the total forty-seven.

TABLE I.

AVOIDANCE OF WIRES.

Myotis lucifugus.

No. of Bat.	Condition Normal.		Eyes Covered.		Ears and Tragi Excised.		Meatus Stopped.		Hair Covered.	
	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.
1	3	8	3	3					7[1]	2
2	9	5	6	6					5[1]	15
3	8	4	1	4			16	21		
4	15	6					19	13	9[2]	8
5	8	7					17	17		
6	8	7					17	18		
7	9	6					18	14		
8	1	3					16	15		
9	8	6			9	5				
10	6	6			15	12				
11	7	5			5					
12	6	3			5	7				
13	8	6			9[3]	5			7	4
14	8	6							15	20
15	7	4								
16	5	3								
Average No.	$7\frac{1}{16}$	$5\frac{5}{16}$	$3\frac{1}{3}$	$4\frac{1}{3}$	$8\frac{3}{5}$	$7\frac{1}{4}$	$17\frac{1}{6}$	$16\frac{2}{6}$	$8\frac{3}{5}$	$9\frac{1}{5}$
Total per cent.		24		15.3		31.4		66.6		36.4

It is therefore apparent that bats deprived of the sense of sight not only are able to avoid objects but, in these experiments, they avoided them better while blinded than they did with sight unimpaired. This does not necessarily mean that they perceived objects more readily with the eyes covered. It was noted that there was a greater tendency to avoid the vicinity of the wires when blinded. The flight seemed slower, although it could not be measured, and more care was probably used to avoid objects.

[1] Eyes freed. [2] Meatus freed. [3] Hair covered first.

The second condition of these experiments, the removal of the ears is also without marked effect on the perception of objects. Five *M. lucifugus* struck the wires 31.7 per cent. of the chances as against 24.4 for the same individuals when normal, and 25 per cent. for all individuals used. Six *M. subulatus* struck 24.6 per cent. of their chances with the ears and tragi removed as against 32.6 per cent. for the same animals when normal. Four *P. subflavus* struck 20.8 per cent. when operated upon and 26 per cent. when normal.

The high percentage of strikes for *M. lucifugus* after the operation is due to a single individual which was injured in the operation. When it is omitted, the percentage for the other four is 24.3, or about that for the normal individuals. The average per cent. for all three species is 23.2 or 1.8 per cent. less than the total average. This difference is so small that it may be accidental and without significance. However, this set of experiments shows that the external ears and tragi are not necessary for the perception of objects. These results are in accordance with the conclusions reached by Rollinat and Trouessart ('00) and Merzbacher ('03).

To stop the external auditory meatus dry plaster of Paris was pressed in lightly with a pair of forceps, and then wet with a drop or two of water. The superfluous water and plaster was wiped out of the ear conch and the bat was held firmly for a few minutes until the mixture hardened. Even then the animals would break the hardened plaster from the ear if they were allowed to rest very long. They were examined at intervals and no trials were recorded in which there was a possibility of the plaster having been removed.

The results were very different from those obtained from the previous experiments. Six *M. lucifugus* struck the wires 67 per cent. of the chances. The same six in the normal condition struck but 26.3 per cent. Five *M. subulatus* struck 65.2 per cent. of chances with ears stopped and the same five normal struck 26 per cent. Five *Pipistrellus subflavus* struck 65.6 times and only 23.2 when normal. The concordance of the figures for these experiments, divided into three groups, is significant and shows that the results are not due to accident but have some common basis.

There are, however, complicating factors which make the interpretation of the results somewhat difficult since the exact way in which the ears are affected is not known. A male *M. lucifugus* used on July 15 seemed to be able to equilibrate perfectly but it flew with a heavy, uncertain flight and was never observed to dodge an object. When it came in contact with any object to which it could secure hold with its claws it clung to it, but always alighted in the position in which it happened to strike instead of reversing in the air and alighting head down. Another bat was able to right itself when tossed end over end into the air but it was never seen to dodge and it struck the wires on 66 per cent. of the trials. Others acted in a similar manner.

TABLE II.

AVOIDANCE OF WIRES.

Myotis subulatus.

No. of Bat.	Condition Normal.		Eyes Covered.		Ears and Tragi Excised.		Meatus Stopped.		Hair Covered.	
	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.
1	5	7					18	20		
2	6	6					16	17		
3	5	11					17	15		
4	9	7					19	16		
5	5	4			1	7	17	8[2]		
6	7	5			9	9[1]			5	9
7	7	5			6	4			14	14[2]
8	6	4			7	5			9	7[2]
9	8	4			8	6			11	12[2]
10	7	8	6	6					7	11
11	9	7	10	6						
12	6	7	8	10						
13	6	5	9	8	5	9				
14	6	6								
Average No.	$6\frac{6}{14}$	$6\frac{2}{14}$	$8\frac{1}{4}$	$7\frac{2}{4}$	6	$6\frac{1}{5}$	$17\frac{2}{5}$	$15\frac{1}{5}$	$9\frac{1}{5}$	$10\frac{3}{5}$
Average per cent.		25.1		31.5		24.6		65.2		39.6

It is difficult to calculate exactly the number of probable strikes if there were no avoidance because the wires were unequally spaced and because the distance between the tip of the wings is

[1] Hair covered.

[2] Ears and tragi removed first.

less on the up and down stroke than when horizontal. The average expanse of the two species of *Myotis* is ten inches. If we deduct one inch for the contracted wings and assume the wires to be equally spaced, the probable percentage of hits is 82. There is therefore some avoidance even when the ears are stopped.

The fact that bats with the meatus plugged were able to equilibrate and alight on objects which they struck would seem to indicate that the disturbance was not a mechanical one, *i. e.*, due to the weight of the plaster or to sensations caused by its pressure on the tympanum or labyrinth, but that it was due wholly to an interference with sensation, and probably to the failure of vibrations to reach the sensory cells of the internal ear.

Table III.

Avoidance of Wires.

Pipistrellus subflavus.

No. of Bat.	Condition Normal.		Eyes Covered.		Ears and Tragi Excised.		Meatus Stopped.		Hair Covered.	
	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.	1st 25 Trials.	2d 25 Trials.
1	5	7					23	19		
2	9	9					17	17		
3	3	6					15	18		
4	6	7					16	15		
5	2	4	2	2			16[2]	17	7[1]	4
6	5	2	5	7					8[2]	9
7	6	4	4	3						
8	7	4	7	4						
9	8	5	6	5					9[2]	5
10	11	13							10	15
11	7	6							8	7
12	8	11			4	8				
13	9	4			5	7				
14	6	7			6					
15	5	2			4	2				
16	3	6			12	11				
17	7	2								
18	5	12								
Average No.	$6\frac{2}{9}$	$6\frac{3}{18}$	$4\frac{4}{5}$	$5\frac{1}{5}$	$6\frac{1}{5}$	7	$15\frac{2}{5}$	$17\frac{2}{5}$	$8\frac{2}{5}$	8
Average per cent.		24.4		20		26.4		65.6		32.8

[1] Eyes and ears freed.

[2] Eyes freed.

Hearing undoubtedly aids the bat to secure the flying insects on which it feeds and thus has been developed to a high degree by natural selection. The perception of a stationary object is probably due to the condensation of the air between the flying bat and the solid body that it is approaching. If hearing is relatively as well developed in a bat as smell is in a dog it is not difficult to imagine that condensation of the air so slight as to be imperceptible to the human ear will arouse sensations on the auditory end organs of the bat. It is reasonably certain that the highly modified external auditory apparatus of a bat has some important function, the exact nature of which is unknown. Flower and Lydekker ('91) state that the function of the tragus is probably to "cause undulations in the waves of sound and so intensify and prolong them." As far as I am aware, no attempt has ever been made to more definitely define the function of that organ. It seems to me highly probable that it also has a selective action, perhaps destroying waves of certain kinds and intensifying others.

It is necessary to bear in mind in discussing the senses of the lower animals that it is impossible to form any adequate conception of the sensations and mental life of the lower animals on the basis of our own. If a piano recital is incomprehensible to a Hottentot, or a snake dance to a cultured Caucasian, how much less can either hope to understand the perceptions aroused in the brain of a hound that scents a fox, or the mental processes of a bat as he circles among the tree tops in pursuit of insects?

The body of a bat is covered with fine hairs of a peculiar structure. The membranes also support hairs, the number varying considerably in the different species. These hairs are supposed to have a sensory function. No means was devised for completely destroying the sense organs located in them without seriously injuring the animals. But they were coated with thick vaseline which pasted the hairs together and made them less sensitive to slight stimulation.

The experiments under these conditions yielded the following results: Five examples of *M. lucifugus* with the hair so coated struck 36.4 per cent. of chances. The same five normal struck 28.8 per cent. Five *M. subulatus* struck 39.6 per cent. of trials

with the hair covered, and 24.4 per cent. when normal. For five *P. subflavus* the proportions were 32.4 per cent. and 25.2 per cent.

The difference of proportion for these three species is considerable and there is no reason apparent. It is not safe, however, to infer that there is any important difference in the sensibility of the hairs of these species for there is a large individual variation, both the lowest and highest individual percentages being found in *Myotis lucifugus*.

The figures indicate that the organs of touch, located in the skin and probably associated with the hairs, are of value in enabling the animals to avoid objects, though of lesser value than the auditory organs. However, it is necessary to take into consideration the mechanical effect of the vaseline in making the wing membranes sticky. Invariably the flight of the bat became more labored, it stopped more frequently and was less readily dislodged from its perch after being covered with the vaseline, although the animals were able to equilibrate and alight on either vertical or horizontal surfaces as well as when in the normal condition.

In order to check the experiments made at the "University Farm" and to determine some points that were overlooked, additional experiments were made in the laboratory at Bloomington, in December, 1907. In place of the wires spaced at irregular intervals, white cotton tapes, 15 millimeters in width, were stretched from floor to ceiling and spaced regularly, the distance between them being 12 inches. The average expanse of *Myotis lucifugus* is 10 inches and there was thus an allowance for error of 2 inches, supposing the bats aimed at the middle of the space. However, the percentage of hits for five individuals, of *M. lucifugus* in 50 trials each was 58.4 when normal and 60 with the eyes covered, as against an average of 25 per cent. in the earlier experiments.

This discrepancy can perhaps be accounted for in part by the method of counting hits. When the wires were used the hits were counted only when audible. With the tapes it was necessary to adopt some other method of counting and a "hit" was recorded every time that the moderately loose tapes were set in motion by

the animal. This could happen without actual contact with the tapes. However, it is improbable that this difference in method alone would account for so great a difference in results. The greater rigidity of the wires would doubtless make them easier to distinguish if the air condensing theory be correct, but not if sight were relied on. The bats would also have less cause to avoid the tapes because striking them would cause no pain. In this set of experiments a distinction was made between "hits" in which the animal struck the tape squarely with the body or upper part of the arm, and "touches" in which the obstruction was merely brushed with the tip of the wing. The preponderance of the latter bears out the assumption that no attempt was made to avoid the objects.

In these experiments each set of fifty trials was divided into five groups of ten each, the object being to see whether there was a progressive decrease of the percentage of hits due to experience. An examination of the table shows that there is no progressive decrease either in the "hits" or "touches," nor for the normal or blinded condition.

TABLE IV.

AVOIDANCE OF TAPES.

Number of Bat.		Condition Normal.					Eyes Covered.				
		1st 10 Trials.	2d 10 Trials.	3d 10 Trials.	4th 10 Trials.	5th 10 Trials	1st 10 Trials	2d 10 Trials	3d 10 Trials.	4th 10 Trials.	5th 10 Trials.
1	Touches.	6	5	9	3	6	6	4	5	5	6
	Hits.	1	2	1	2	6	2	4	0	3	2
2	Touches.	5	7	5	4	2	5	3	2	2	2
	Hits.	3	0	0	2	0	0	1	1	2	2
3	Touches.	6	7	3	3	9	4	3	2	7	4
	Hits.	0	1	2	2	0	2	1	2	0	2
4	Touches.	6	7	5	7	4	7	9	10	8	9
	Hits.	1	1	1	0	0	0	0	0	1	0
5	Touches.	3	2	2	3	2	6	3	1	5	4
	Hits.	1	2	1	1	1	1	2	0	0	0
Average No. of Touches.		$5\frac{1}{5}$	$5\frac{3}{5}$	$4\frac{1}{5}$	4	$4\frac{3}{5}$	$5\frac{3}{5}$	$4\frac{2}{5}$		$5\frac{2}{5}$	5
Average No. of Hits.		$1\frac{1}{5}$	$1\frac{1}{5}$	1	$1\frac{2}{5}$	$1\frac{2}{5}$	1	$1\frac{2}{5}$	$4\frac{3}{5}$	$1\frac{1}{5}$	$1\frac{1}{5}$
Average of both.		$6\frac{2}{5}$	$6\frac{4}{5}$	$5\frac{1}{5}$	$5\frac{2}{5}$	6	$6\frac{3}{5}$	6	$4\frac{3}{5}$	$6\frac{3}{5}$	$6\frac{1}{5}$

Per cent. of totals for normal condition, 60.8.

Per cent. of totals for blinded condition, 60.

To determine whether there was any avoidance or whether the animals hit or missed by accident, a ball of cotton with one diameter equal to the expanse of the bat and the other slightly smaller, to compensate for the upward stroke of the bat's wing when the distance from tip to tip is somewhat less, was thrown at random at the tapes. The ball struck 82 per cent. of the trials, or approximately the calculated number, as against the maximum of 60 per cent. for the bats.

The difference is more apparent when we separate the "hits" from the "touches." For the ball the "hits" were 48 per cent. of the total chances and "touches" were 34 per cent. For the animals the percentage of "touches" in the normal condition is 48.4 and 49 with sight eliminated. On the other hand, the "hits" were 9.6 per cent. of the chances under normal conditions and 16.6 per cent. with the eyes covered. From these figures it is apparent that the animals avoid striking objects in such a way as to impede their flight much more often than they avoid brushing against them with the tips of the wings.

In the caves I have often seen horizontal scratches on mud banks or on slime-covered walls that must have been made by flying bats that were unable to completely avoid the obstacles in their path. I have not seen any evidence that they ever strike the walls hard enough to do themselves injury. The great agility with which a bat can check its flight or change its course enables it to either turn aside or take hold of an object which it strikes even if it is not perceived until the animal is almost against it. It is highly probable that the fatty pads which lie about the nostrils have a protective value and prevent injury to the animal when it strikes, head on.

The experiments described above show that bats do not always avoid obstacles in their path. Spallanzani's statement as to the accuracy with which they perceive objects in their pathway, on which a number of writers on natural history have based erroneous statements, are incorrect, at least in so far as they apply to the species studied in the preparation of this paper. On the other hand, these experiments show that bats do perceive objects that they are approaching by senses other than sight or hearing as usually understood. The most important sense organs for the

perception of objects are in the internal ear. The hairs of the body and membranes also have a sensory function. The external ears, the tragi and the eyes are not necessary for the guidance of the animals, although there is reason to believe that when they are flying in the light they depend, to some extent, upon the sense of sight to perceive objects.

Additional Observations.

A large brown bat, *Eptesicus fuscus*, brought into the experiment room May 2 seemed wholly unable to avoid the wires. It flew rapidly, was not seen to dodge any obstacle, and struck the wires 67 times out of 100 chances when uninjured. It appeared to be frightened by its unusual surroundings.

A long eared bat, *Corynorhinus macrotis*, captured May 1, struck 52 times out of 102 chances. After it had been flying in the room for ten or fifteen minutes it began flying against the windows. It returned to the same point time and again, striking the pane when the window was closed or the wire screen, if the window was open. Usually it struck with considerable force and fell to the sill, but immediately got up and repeated the performance. An adult *Myotis lucifugus* liberated in the house on April 30 acted in the same way and other individuals of both the common species of *Myotis* flew against the glass and window screen.

There were great and unaccountable individual differences in this regard. A male *M. subulatus* on September 3 struck the screen repeatedly, both when the eyes were normal and when they were blindfolded. Another male of the same species used on the following day flew directly toward the screen a number of times but always turned in time to avoid it. Apparently in these instances the animals were depending upon the sense of sight in guiding their movements. The window glass would be invisible to an animal that had never had experience with transparent objects and the wire screen was not very apparent against the background of trees among which the house is situated. The actions of the bats in flying about the room at certain times seemed to indicate that they were depending on sight for guidance in avoiding the wires. The flight when the eyes were cov-

ered was usually slower and more cautious than when the senses were unimpaired.

In the cases where they flew against the screen that obstructed the open window, the bats may have been attracted by incoming currents of air. In experimenting with them in a closed room they almost invariably found the cracks under doors, in the sides of the room, or under the roof and the experiments were seriously delayed by a large number of the animals escaping through crevices which were overlooked or were supposed to be too small for the passage of their bodies. They always explore every corner of any compartment into which they are placed and their manner often indicates that they are attracted to an opening from a distance of several feet when the air currents are the apparent stimulus.

II. *The Formation of Associations and the Sense of Direction.*

The experiments described in this section deal chiefly with a single kind of association, namely, that of place. In studying this sort of association, data were obtained which seem to indicate the presence of a sense of direction not based directly on any of the five senses commonly recognized.

The peculiar habits of a bat make it impossible to employ the methods generally used by animal psychologists in studying the formation of associations. Bats will not go to a dish for food at regular intervals. Although they readily learn to escape from any possible opening, they do not have any adaptation for grasping which would enable them to learn to pull a string or raise a latch and so open a door.

I did not find any evidence that associations of form or color are ever formed. Such associations are hardly to be expected in animals with visual organs so poorly developed.

Sound associations are formed readily. A sucking noise made by the lips at first alarmed the animals, but they soon learned to associate it with feeding. On hearing it they would look about and snap at any object that could be mistaken for food. One individual (bat No. 2 mentioned below) was especially quick to form this association and learned to come on hearing the sound, although it did not learn to localize it definitely. Alcock ('99)

states that a hairy-armed bat, *Vesperugo leisleri* (*Pterygistes leisleri*), learned to come for food on hearing a pair of scissors clicked together.

For studying place associations the following method was used: The bats were kept in cages in the dark room as previously described. For the experiments they were taken into a well lighted room and placed in a small experimental cage made of wire cloth, the sides being of one fourth inch mesh and the top, bottom and ends of one eighth inch mesh. The dimensions were 12 by 13 by 27 inches. On one side was cut a hole seven inches square. This opening was closed with a door made of the same material as the side, and overlapping the edges an inch all around. It was fastened with a wooden latch on the outside. A piece of white cloth, three inches square, was fastened inside the cage, near the upper left corner of the door.

The bat to be used in the experiment was placed on the floor of the cage near the middle. All of its movements were carefully recorded during the whole time it was in the cage. As soon as it touched the cloth while following its natural tendency to explore every part of the cage, the door was opened and a meal worm was offered it with a pair of forceps.

Animals that had never been handled were usually frightened away by thrusting the hand toward them and moreover they did not know how to eat the meal worms. Therefore it was necessary to use bats that had been in captivity for some time and had learned to eat the food offered them.

As soon as the animal under observation had eaten the food given it, it was again placed on the bottom of the cage and given another chance to come to the same place for a worm. The time required was carefully noted and also the movements of the animal which did not result in bringing it nearer to the food.

The curves given by Porter ('04) for similar observations on English sparrows, and Kinnaman ('02) for monkeys, are fairly uniform after the animal had found the food once or twice. The animals used by these observers apparently responded in about the same way to the same stimulus in all instances where there was no disturbing factor. The reactions of a bat are much less constant. When placed in the experimental cage it sometimes

goes at once to the spot where food is given. At other times when it should be about as hungry, it sits quietly on the floor for five minutes or longer and then goes without error or hesitation for the food. Even when it wanders about the cage instead of going directly to the feeding place, it cannot be asserted that the animal has forgotten where it must go for food; the impulse to explore the cage may be stronger than the hunger impulse.

The erratic behavior of bats makes it impossible to tabulate the results or to plot a curve of the time of response that will give a correct idea of the behavior of the animal. For this reason the record of the observations for one bat will first be given in considerable detail, and the conclusions will be stated afterward.

This bat, a female *Myotis subulatus*, recorded as No. 2 in my experiments, was obtained in Shawnee Cave at Mitchell on December 8, 1907. It was kept in a small cage with other bats in the dark room and was occasionally taken out and fed meal worms and allowed to fly about the laboratory. It could always be easily aroused from its dormant state and was unusually alert and active.

In the following records it is to be understood, unless otherwise stated, that the time recorded is that from the instant the animal was released in the middle of the cage until it touched the cloth.

This bat was first placed in the experimental cage on February 7, at 2:06 p. m. (1) It ran and flew about in all parts of the cage and in three minutes reached the cloth and took it in its teeth, probably mistaking it for food because it moved when touched. (2) Was fed and remained quiet for a time, then left and came back and was fed at 2:27. (3) Put on bottom of cage and came back in 1½ minutes but left before it could be fed. (4) Back and fed 3 minutes later. Crawled away and became quiet and was taken out.

Was not put in again till February 10, at 3:39. First time came to cloth in 6 minutes; second time in 1½; third in 1; fourth in 2; fifth in 70 seconds; then in 40 seconds. Experiment terminated.

It is evident that the association had been definitely formed at this time or after a total of ten trials, the first four of which occurred three days earlier than the last six.

February 11: (1) Put on floor of cage at 3:25. Flew up to cloth in 20 seconds. (2) Flew up to front, went across to cloth and began pulling at it in 17 seconds. (3) Flew directly to cloth after ten seconds. (4) Looked about, flew to right end of front, then ran to cloth in 30 seconds. (5) Hesitated and looked about, then flew to front and reached cloth in 30 seconds. (6) Was quiet, then turned and flew directly to cloth in 40 seconds. (7) Went to corner of cage, hesitated, then flew to front and went directly to cloth in 35 seconds. (8) Flew directly to cloth in 10 seconds. (9) Did not move for 75 seconds, then turned partly around and flew directly to cloth. (10) Looked around, scratched itself and washed its face, then after 2½ minutes, flew without hesitation to cloth.

Was not taken out of the living cage on February 12.

February 13: (1) Put in cage at 3:58½. Flew to end of cage and climbed across to cloth in one minute. (2) Flew to right front, walked across and was fed in 20 seconds. (3) Looked around and flew directly to cloth in 12 seconds. (4) Turned around several times, flew into corner and went to cloth in 45 seconds. (5) Was quiet an instant, turned and flew to cloth in 45 seconds. (6) Put on floor, flew to front of cage near cloth, turned toward it but stopped, looked about, then cleaned its fur and became quiet; nearly five minutes after being put on the floor it again began to look about, then went directly to cloth and was fed. (7) At once ran to front of cage and climbed it but did not go to cloth till 3 minutes later. (8) Remained quiet a minute, then climbed up in corner nearest cloth and rested a minute, then went directly to cloth. (9) Remained quiet on floor for a minute, then flew to end of cage and remained for some time, when the experiment was discontinued.

February 14: There were no peculiarities in its activity. The times for the trials were as follows: (1) 15 seconds; (2) 20 seconds; (3) 10 seconds; (4) 7 seconds; (5) 20 seconds; (6) 30 seconds; (7) 12 seconds; (8) 45 seconds; (9) 45 seconds; (10) failure, the animal settled at one end of the cage and remained there until taken out.

Was not put in the experimental cage nor fed on the fifteenth, sixteenth, or seventeenth.

February 18: (1) Put in at 2:47 and flew about the cage, bumping the sides; rested on the bottom, then flew to front and went to cloth in 4½ minutes. (2) Put on floor, rested on end of cage, then went to cloth in 6¼ minutes. (3) Flew to front and went across to cloth in 20 seconds. (4) Flew to door of cage in 10 seconds, but seemed to have learned that food came in through door and waited there. Got to cloth in one minute. (5) Sat on floor without moving for 2 minutes, then flew directly to cloth but started across to edge of door before it could be fed. (6) Was quiet 1 minute, then flew directly to front of cage and reached cloth in 70 seconds but again turned to door. Was put back on floor without being fed, started away but came back and began chewing cloth and was fed 2½ minutes after being first put on the floor. (7) Remained quiet 3 minutes, then flew to front and started toward cloth but stopped and cleaned its fur, remaining there 15 minutes; then turned suddenly and went directly to cloth. (8) Remained quiet 1 minute, then flew directly to cloth. (9) Remained on floor for 10 minutes and then was taken out.

February 19: (1) Put in at 2:05, seemed rather torpid; walked across cage, then remained quiet until 2:13 when it flew directly to cloth. (2) Flew to front of cage and started to cloth in 45 seconds but stopped, 20 seconds later went to it but heard my hand at door and started to it; was not fed; 1 minute later, turned and went again to cloth and was fed. (3) Remained quiet, then flew to front and went directly to cloth and pulled at it with its teeth in 2½ minutes. (4) Was quiet, then flew directly to front and went to cloth in 1 minute. Was beginning to turn toward door again when fed. (5) Cleaned its fur and was quiet, then flew directly to cloth in 4½ minutes. (6) Was quiet, then flew directly to cloth after 7 minutes. (7) Quiet, flew directly to cloth after 3 minutes. (8) Quiet, flew directly to cloth after 2 minutes. (9) Quiet, flew to front and went directly to cloth in 1¾ minutes. (10) Quiet, flew to front near cloth in 2 minutes, but remained there cleaning fur 6 minutes longer when it turned and began to pull cloth with its teeth. (11) Flew directly to cloth after 1 minute. Taken out.

For February 20 the times are: (1) 1¼ minutes; (2) 15 sec-

onds; (3) 2½ minutes. (4) Quiet 12 minutes, was disturbed and then went to the cloth in 2 minutes. (5) Quiet 5 minutes, disturbed, then responded in 1 minute. (6) Quiet 11 minutes; disturbed, then went to cloth in 3 minutes. (7) 45 seconds. (8) 8 seconds. (9) 75 seconds. (10) 45 seconds.

February 21: (1) 75 seconds; (2) 45 seconds; (3) 1½ minutes. (4) Became quiet for 8½ minutes; disturbed, then went to cloth in 1 minute; (5) seven seconds; (6) 12 seconds. (7) Quiet for 5 minutes. Disturbed, then went slowly to cloth in 1 minute. (8) Quiet; disturbed, then became quiet again and was disturbed a second time. Went by indirect route to the cloth 2½ minutes after second disturbance. (9) Ran to front of cage and climbed to cloth in 20 seconds. Dropped the worm given it and then turned around and took hold of the cloth with its teeth. (10) Became quiet; was disturbed after 7 minutes and flew directly to cloth 25 seconds later.

February 22: (1) Put in at 1:57. Flew directly to cloth and pulled at it with teeth in 10 seconds. (2) Flew directly to cloth in 15 seconds. These two trials show that, as on several preceding days, there was no error in finding the cloth. There has been delay due to inhibition of the stimulus or the lethargy of the animal but it has been finding the piece of cloth quickly whenever it was trying to find it.

At this point the bat was taken out of the cage and placed temporarily in a box. The cage was rotated through 180 degrees so that the front now faced the west instead of the east. The observer's chair was also moved to the west side and a box in which there was another bat moving about was moved from the east side where it had been kept during the greater part of the experiments, to the west side. It is to be remembered that the door of the cage is in the middle upper part of the front and the cloth is at the upper, left or back edge of the door, 7 inches from the left end of the cage and 20 inches from the right end.

After the cage was reversed and the observer again seated in front, the bat was placed in it.

Trial (1). — Was placed on the floor facing the cloth; looked about, slowly turning its head, then turned the body and *flew directly to back of cage at a point about 7 inches from the right end*

or the same absolute spot that it had been accustomed to go to, but a place diagonally across the cage from the piece of cloth. Remained there for 7 minutes. (2) Put on floor facing cloth again and looked about, then turned and flew to same point in back in 50 seconds. Seemed to be looking about for cloth, then became quiet. (3) Again put on floor facing cloth, turned and flew to middle of back in 75 seconds and climbed all around over that part of the back of cage. (4) Remained quiet 4 minutes, then turned and flew to same spot as last time ; looked and crawled about, then became quiet. (5) Flew to back in 70 seconds and settled down without crawling about. (6) Flew to back in 75 seconds, looked about very little, then became quiet. (7) Put on floor very near cloth, was quiet, then flew directly to back in 60 seconds ; climbed and looked around all over back before settling down. (8) Looked around, then turned and flew directly to back, climbed about on back and then became quiet. (9) Remained quietly on floor, then flew to back in 2½ minutes and climbed about over it. Flew to lower right front (possibly attracted by squeakings of another bat in a box near there). Was quiet, then went to back of cage again and climbed about over that ; it finally flew to right front corner and from there to the floor where it rested. (10) Flew to back at right of middle and became quiet.

At this point in the experiment the bat was taken out and the cage was turned to its original position. (1) The bat was again placed in the middle of the floor and crossed back and forth several times, then flew to front near cloth in 75 seconds and began pulling at it violently. (2) Flew to cloth in 5 seconds. (3) Flew to cloth in 20 seconds. (4) Flew to cloth in 12 seconds. (5) Flew to cloth in 25 seconds. (6) Remained quiet on the floor, then flew to cloth in 1½ seconds. (7) Flew near cloth and went to it in 30 seconds. (8) Flew near cloth and went to it in 50 seconds.

On the following day the bat was again placed in the cage in its original position, *i. e.*, the front to the east, and on the ten trials of this day it went quickly and without error to the cloth. Before any other experiments could be made with this animal, it escaped from its cage and could not be recaptured.

Four other bats were used in the same kind of experiments between February 7 and March 6. The details of these experiments are, in general, similar to those outlined above. Each of the bats died before the observations were completed.

Bats numbers 4 and 5 both of which were female *Myotis lucifugus*, were used with a piece of bright carmine-red cloth, four by five inches square, in the cage instead of the smaller piece of white cloth. In the case of bat No. 4 the cage was reversed on the second day, or after the animal had been fed at the cloth only 13 times. The association had been quite firmly fixed, however, and the bat went to the back of the cage eight successive times after it had been reversed before it wandered about sufficiently to find the cloth. When it did get to it, it seemed to remember the place and took the cloth in its teeth. It was fed here five times but showed some confusion in finding the place and several times went to the back. The next day it also appeared confused when first placed in the cage and sometimes went to the cloth and sometimes to the back. The following day it seemed to be sick and died two days later.

Results of the Experiments on Association and the Sense of Direction.

These experiments show that visual associations are formed slowly or not at all. Sound associations are formed more readily. Tactile associations were not isolated from others but probably enter into the perceptions which lead to finding the cloth as the animals seemed to have the cloth, as well as the location of it, associated with obtaining food.

The facts relating to the sense of direction will now be taken up. The bats were fed meal worms while they rested on a piece of cloth. The cloth became soaked with the juices of the worms and it also acquired the characteristic odor of the bats to a sufficient degree for the human nose to detect it. The bats did not rely upon the sense of smell for finding the place or they would have reached it without error.

The room in which the experiments were conducted was not as free from noise as might have been desired. However, it was a basement room with thick walls and there was generally no one

except myself in the room or adjoining halls, and outside noises were not heard to a large extent. The only noises recurring with any degree of regularity in the room were those made by the steam in the heating pipes. These could have no direct association with the giving of food while the movements of the observer near at hand did have such an association and these were perceived by several of the animals at times, as when they left the cloth and started toward the sound of the opening door. Therefore sound cannot be considered as a factor in guiding the bats to the back or front of the cage.

Taste and touch may also be counted out, because, if they entered into the food associations at all, they would each tend to guide the animal toward the cloth.

It is not possible to say with so much assurance that sight is not a factor. It was possible for the animals to look through the sides of the cage, but there were no conspicuous objects near and the door of the cage with its latch, and the white or red cloth, were much more noticeable than anything else in sight.

In describing the action of the bat in the cage I have said in a number of places that it "looked around," but it is not certain that this action was really for the purpose of seeing. In a number of trials the animals were so placed that they faced the cloth and if they had been looking for familiar objects as landmarks to guide them to the food they would have noticed it first of all and would have gone directly to it.

The only way in which it seems possible that sight could have aided in their orientation is through the direction of the rays of light. This is not probable because the room was well lighted by windows on two sides and the experimental cage always stood back out of the direct sunlight and also out of the shadows. Moreover, we should not expect to find that animals accustomed to spending all their lives in total darkness or twilight, would depend upon the visual sense for orientation.

While it must be admitted that the experiments did not exclude every possibility of some effective sensations being received through the five senses we commonly know, yet it is not possible to fully account for the behavior of the animals in the experiments above described on the basis of these senses alone, unless

they are developed to a degree which we know nothing about from direct experience.

Watson ('07) found that the ability of white rats to learn a maze was not impaired by the destruction of either the eyes, the olfactory lobes, the middle ear or the vibrissæ, or by anesthetizing the paws or nose, or eliminating temperature and air currents. When the maze was rotated through an angle of 90 degrees, they were confused, but when it was rotated through 180 degrees they were again able to find their way. Watson believes that "static sensations or some non-human modality of sensation" are necessary to explain the behavior of these rats.

Watson and Carr ('08) believe that in the white rats, orientation is attained by traversing a unit of the maze. In man, a train of acts which have become habitual may be set off by some visual or other sensory impulse. In the lower animals such acts may be set off by a "kinæsthetic sensation," such as traversing a unit of the maze and getting the appropriate "feel" of direction from some combination of motor impulses or acts.

Bats are more difficult to work with than rats because their reactions, always uncertain, are seriously disturbed by any kind of operation. Those that I have had have also lacked vitality and have died before extensive experiments could be completed. However, the experiments here described seem to warrant the assumption that they also have something akin to "static sensations" which enables them to retrace their way to a point at which they have been before, without depending on the other five senses.

It is not the purpose of the present paper to discuss the nature of this sense. It may be the same as the "kinæsthetic" sense of Watson. However it is not necessary for a bat to perform an act similar to that of the rats traversing a unit of the maze in order to obtain orientation. The only movements of the bats which seemed to have any connection with orienting was a slow turning of the head in various directions. The purpose of this I could not determine. It is conceivable that if the sense of location is situated in the semicircular canals, a rotation of the head might arouse various sensations, one of which would serve as a clue to position.

There is reason to believe that bats have good memory. On

one occasion a male, *M. lucifugus*, that I had marked by excising the right tragus, escaped through a small crack under the door into another room and thence to the outside through one of several small holes. A few days later I found it in the cave and brought it to the room again, and liberated it.

It circled twice about the room and then dropped to the floor near the door and started directly for the crack and escaped from the house before I had a chance to stop it. Several days later it was recaptured a second time and turned loose in the same room. It started at once for the crack under the door. The crack had now been stopped so that it could not get out, but it ran about in that corner and for several days, whenever liberated in the room it repeatedly went to the place where it had escaped before.

Certain bats, released in a room, show preference for alighting in a particular spot while other individuals select other spots. To illustrate, two bats were allowed to fly about a room lighted by seven windows, all of which received about equally strong light. Each bat alighted a number of times. One of them selected the casing of window number two 12 per cent. of the times and all other windows 12 per cent. Another bat selected window number five 28 per cent. of the times and did not go to window number two at all. Other instances could be cited illustrating the same point. The bats apparently find a place suitable for resting by accident the first time and later return to it because it is remembered.

In experimenting with the bats in the cage, I found that they also learned by accident to find the place where food was to be obtained. When food was once associated with a certain place the animals very quickly learned to go back there. After they once learned the association it remained very persistently. In the experiments with bat No. 2, outlined above, it was so persistent that it prevented finding a new place in ten times the number of trials required the first time.

Memory in all of these cases is doubtless below the realm of consciousness and akin to that which in man is rendered subconscious through habit. Some sort of a memory is absolutely necessary in order to make a sense of direction of any value to

the animal. It is necessary that a bat "remember" the points at which it has been or a sense of direction would not help to orient it.

The sense of direction in these bats may very probably be accounted for, at least in part, by the high development of associative memory. A man can learn to go about a house and make all the turns correctly through habit and with little or no dependence on his senses. In his case it has probably required long experience and many repetitions of the act. In the bats, an act is learned very much more quickly and it is possible that one or two repetitions may even be sufficient to render the performance automatic. If this is true, the ability of the animals to find a place at which they have once been, may be based neither on a sixth sense, nor directly upon any of the five senses, but upon associative memory and quickness in forming habit.

The utility of a sense of direction to bats is so apparent as to scarcely require discussion. It is impossible for sight to be of any service in helping them to find their way in the caves. Outside noises do not go in far and few noises originate there, so that hearing can be of little service in orienting them.

It has been suggested (Blatchley, '96) that air currents may guide them to the mouth of a cave, but this is to me inconceivable. Not only does the direction of the current change in the principal passages but there are always eddies in the chambers and tortuous passages which would tend to confuse rather than help them. The only odors are the constant ones characteristic of a cave and since the bats pass through the air and not along solid surfaces their own odor is not left with a sufficient degree of permanence to be of service in guiding them.

But if a bat have a sense of direction well enough developed to guide it in retracing its way, it would have an immense advantage over other animals of similar habits that lack such a sense. Thus natural selection would foster and improve it.

Conclusions.

Bats are separated from all other mammals by a number of morphological peculiarities which are correlated with the adaptation for flight.

They have no nests, dens or fixed homes. The species studied stay in the caves during the greater part of their existence. They usually go in far enough to be in a constant temperature and total darkness but do not select their resting places with reference to the size of the cave, the nature of the opening or the amount of moisture.

They have few enemies. Consequently fear is but little developed.

About five sixths of a bat's entire existence is spent in a dormant condition. This condition is not dependent upon temperature or season but upon the condition of metabolism; a large amount of fat is favorable to torpor.

In the caves, where conditions of light and temperature are constant, bats come to the cave entrance at irregular intervals. The length of time between these intervals depends upon the amount of surplus fat stored in the body.

They leave the cave only when favorable conditions of light and temperature prevail, and go back to the interior of the cave if the light is too intense or the air too cold.

Food consists of insects that are caught on the wing.

Several senses aid in its perception. Smell and taste are of no use for this purpose. Sight may aid to some extent. Hearing and the tactile sense are chiefly relied on to perceive and locate food.

Bats are more helpless on their feet than most birds. In the air they have greater agility.

They can check their momentum very quickly. In flight, they can secure hold of a surface, only slightly rough, with a single thumb or with one foot.

The breeding habits of our species are not well known. They mate in the fall and the young are born early in the summer. Breeding females leave the caves during the period of gestation and rearing the young.

The sexes do not segregate while they remain in the cave.

Bats in captivity do not readily learn to pick up food from the floor of the cage. They will eat food presented to their mouths and will go to a dish for water.

They do not live well in captivity except when in the quiescent state.

Experimental studies show that neither sight nor the external ears and tragi are necessary for the perception of obstacles during flight.

The body hairs probably have a sensory function.

Obstacles are perceived chiefly through sense organs located in the internal ear.

Perception is probably due to the condensation of the atmosphere between the moving animal and the object it is approaching.

Bats show a remarkable ability to return to a particular spot for food or for the purpose of escaping from an enclosure.

It is difficult to explain how they find their way by means of the five senses familiar to us.

The presence of a sixth sense, that of direction, will explain all of the facts.

It has not been conclusively shown that such a sense exists. If it exists in any animals we should expect to find it in bats. Their habits are such that a sense of direction would be of advantage to them in the struggle for existence.

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ON THE RELATION OF RACE CROSSING TO THE SEX RATIO.

MAUD DEWITT PEARL AND RAYMOND PEARL.

INTRODUCTION.

There would appear to be widely prevalent among practical stock breeders an opinion that the relative proportion of the sexes may be influenced by the method of breeding practiced. As evidence of the existence of such an opinion two citations will suffice. Others might be given. Davenport in his memoir on "Inheritance in Poultry,"[1] introduces a section on "Sex in Hybrids" (p. 97) with the statement that: "There is a widely held and frequently expressed opinion that hybrids show an excessive proportion of males." He further says that: "Bateson and Saunders probably have this in mind in their statement — 'the statistical distribution of sex among first crosses shows great departure from the normal proportions.'" No support is given to the view that hybrids show an undue proportion of males by Davenport's own statistics, the general conclusion being that: "The exceptions to the law of equality of sexes in hybrid offspring are . . . individual and not of general significance."

It is a matter of interest to note that while the opinion appears to be widespread that the kind of breeding practiced influences the sex ratio there is not entire uniformity as to what the influence of a particular method of breeding on sex is. Thus one would infer from a statement in a recent work by Müller[2] that it has been generally held by continental breeders, at least, that *inbreeding* tends toward the production of an unduly large proportion of males. Müller[2] (*loc. cit.*) in discussing the experiments of Schultze (*cf. infra*) makes the following statement concerning certain of that author's results: "Das Verhältnis der beiden Geschlechter war vielmehr bei strengster Inzucht (Paarung nur

[1] Carnegie Institution of Washington, Publication No. 52, 1906.

[2] Müller, R. "Biologie und Tierzucht." Gedanken und Tatsachen zur biologischen Weiterentwicklung der landwirtschaftlichen Tierzucht. Stuttgart (Ferd. Enke), 1905. Pp. 96.

mit Bruder, Enkel, Urenkel, Vater und Grossvater) ein sehr verschiedenes, ja in einigen Fällen kamen sogar in der dritten Geschlechtsfolge, *ganz in Gegensatze zu der alteren Annahme*,[1] überwiegend weibliche Nachkommen zur Welt."

Investigations systematically directed towards determining in what way and to what extent either hybridizing or inbreeding affect the sex ratio are very few in number. Davenport (*loc. cit*), from a tabulation of the sex of 377 fowls reaches the conclusion already stated regarding the influence of hybridization. Schultze[2] has studied in mice the effect of inbreeding of various degrees including the closest "Inzestzucht" on sex determination, and reaches the conclusion that in general it has no effect.

The search for factors which may determine or influence sex is being actively prosecuted by experimental biologists. Any data tending to throw light on the significance of any supposed sex-influencing factors can but be welcome. The quotations from the literature which have been given suffice to indicate that the character of a mating must at least be accorded the place of a "supposed" sex-influencing factor. It is the purpose of the present paper to exhibit and discuss certain data which have a direct and definite bearing on the question of the significance of this factor in the case of one organism, namely, man.

The data which form the basis of this paper are extracted from the published vital statistics of the city of Buénos Ayres. For nearly twenty years past this city has maintained an elaborate system of municipal statistics. Indeed its system might in many respects well serve as a model. It is doubtful whether the statistics of any other city or country surpass those of Buénos Ayres in completeness and accuracy. These records are published in annual volumes, of which fifteen have appeared. The statistics of births given in these volumes are particularly detailed. Among other matters of general biological interest there is given each year a table setting forth the number of births occurring in the year covered by the volume, classified in such way that it appears for each child born whether it was (*a*) male or female, (*b*) legiti-

[1] My italics. — R. P.

[2] Schultze, O. "Zur Frage der geschlechtsbildenden Ursachen." *Arch. mikr. Anat.*, Bd. 63, Heft. 1, 1903.

mate or illegitimate, and (*c*) what was the nationality of each of its parents. Furthermore it should be said of these statistics that they are *registration* figures and not *census* returns. That is to say, they are definite *records* of events, each event being recorded when it happens, not more or less inaccurate counts made a long time after the event. Of the substantial accuracy of these figures there can be no doubt.

As is well known, Buénos Ayres is a city having a population which is racially very heterogeneous. For a decade and more past there has been a large Italian immigration. Also there has been extensive Spanish immigration. Representatives from other nations have come in in smaller numbers. From the statistics of birth above alluded to it is possible to determine what has been the sex of the offspring of each of these racial groups in pure matings and when crossed with native Argentine stocks. For the purpose of the present study the birth statistics of the ten years 1896–1905 inclusive have been used. The following matings have been considered:

Argentine	♂	Argentine	♀
Italian	♂	Italian	♀
Spanish	♂	Spanish	♀
Italian	♂	Argentine	♀
Spanish	♂	Argentine	♀

Data are available for other matings but it has not seemed advisable to deal with any yielding less than 8,000 offspring in the ten years. The inquiry has been further limited to legitimate births, because of the uncertainty which must always exist in the great majority of illegitimate births as to whether the putative father is the actual one. With these restrictions the number of separate offspring dealt with in this study approaches a quarter of a million (exactly 219,516).

These statistics have been studied with the purpose of obtaining answers to the following questions:

1. Is there a tendency towards an excessive production of offspring of one sex (either male or female) in cross as compared with pure matings, among the human racial stocks under consideration?

2. If such a tendency appears to exist is it (*a*) uniformly shown in all the matings considered, and (*b*) numerically great enough in amount to be considered significant when tested by probable errors ?

Data.

The raw material on which this paper is based is set forth in Table I. The figures are extracted from Volumes VI. to XV. inclusive of the *Annuaire statistique de la ville de Buénos-Ayres.*[1]

Table I.

Sex Distribution of Legitimate Births. Raw Data.

Nationality of Parents.	Argentine ♂ / Argentine ♀		Italian ♂ / Italian ♀		Spanish ♂ / Spanish ♀		Italian ♂ / Argentine ♀		Spanish ♂ / Argentine ♀	
Year.	♂♂ Born.	♀♀ Born.	♂♂ Born.	♀♀ Born.	♂♂ Born.	♀♀ Born.	♂♂ Born.	♀♀ Born.	♂♂ Born.	♀♀ Born.
1896	1,597	1,654	5,326	5,455	1,814	1,695	939	932	349	411
1897	1,722	1,712	5,740	5,499	1,767	1,728	1,060	968	431	394
1898	1,922	1,773	5,765	5,703	1,805	1,695	1,152	984	431	420
1899	1,980	1,945	5,770	5,743	1,887	1,790	1,168	1,100	478	417
1900	2,038	1,950	5,070	5,620	1,809	1,784	1,126	1,064	458	411
1901	2,163	2,099	5,923	5,771	1,879	1,806	1,178	1,192	461	421
1902	2,189	2,100	5,736	5,597	1,837	1,781	1,204	1,189	463	438
1903	2,277	2,200	5,341	5,133	1,780	1,702	1,214	1,127	467	448
1904	2,352	2,368	5,419	5,240	1,952	1,723	1,345	1,215	521	460
1905	2,533	2,317	5,507	5,409	2,093	1,940	1,294	1,277	516	468
Totals	20,773	20,118	55,597	55,170	18,623	17,644	11,680	11,048	4,575	4,288

It is at once apparent that these statistics show essentially the same relation of the sexes as that usually found when large numbers of human births are examined, namely, a preponderance of males. The extent of this preponderance may be shown best by putting the data in the form of sex-ratios. In this paper the sex ratio will be taken as the number of males to each 100 females. The sex ratios deduced from the totals of Table I. and their probable errors are given in Table II. It does not appear to be necessary or advisable to deal with the single years separately. The method of determining the probable errors of the sex-ratios was to determine first for each mating the probable error of the absolute frequency of males, considering this as a simple class

[1] Published by the Direction générale de la statistique municipale, Buénos Ayres.

frequency. It has been shown[1] that if y_s be any class frequency within a sample containing m individuals altogether, then

$$P.E._{y_s} = .67449 \sqrt{y_s \left(1 - \frac{y_s}{m}\right)}.$$

From the "absolute" probable error so obtained the probable error of the sex-ratio is easily deduced.

TABLE II.

MALES TO 100 FEMALES FROM TOTALS OF TABLE I.

Mating.	Sex Ratio.	Mating.	Sex Ratio.
Argentine ♂ Argentine ♀	103.26 ± .34	Italian ♂ Argentine ♀	105.72 ± .46
Italian ♂ Italian ♀	100.77 ± .20	Spanish ♂ Argentine ♀	106.69 ± .74
Spanish ♂ Spanish ♀	105.55 ± .36		

From this table the following points are to be noted :

1. The number of males to 100 females varies between approximately 101 and 107 in the different matings. There is an excess of males in every case. Further, this excess is significant in amount as is indicated by the probable errors. The present statistics agree with other large collections of data regarding the sex-ratio of human births. There appears to be no doubt that a tendency towards the production of a greater number of males than of females is normal for Caucasian races at least.

2. The sex-ratio is in each case higher for the cross matings than for the pure. That is, there are more males per hundred females produced when the parents are of different racial stocks than when they are of the same.

The answer to the first question propounded above (p. 196) then is that there is a definite tendency towards an excessive production of male offspring in cross as compared with pure matings in the data here considered. Further, it appears that within the limits of the present material this tendency is uniformly exhibited in all the matings.

Attention may next be turned to the second part of the second question, which may now be put as follows :

[1] Editorial — "The Probable Errors of Frequency Constants," *Biometrika*, Vol. II., p. 274.

Is the excess of male births in cross matings numerically great enough to be considered significant in comparison with the probable errors involved? The evidence on this point is presented in Table III., which compares the sex-ratio for each cross mating with that for each of the two pure matings related to it. The last column of the table gives the ratio of the difference in each case to the probable error of the difference. In interpreting this last column it will be remembered that a difference which is three or more times as large as its probable error is to be regarded as significant; a difference which is between two and three times its probable error is probably significant; while a difference less than twice its probable error when taken by itself is probably not significant. In general, the technical biometrical use of the term "significant" intends to convey the idea that the odds are so great as to amount to practical certainty that a so-called "significant" result did not arise simply as a purely chance effect of random sampling, but represents a direct causal nexus between phenomena.

TABLE III.

COMPARISON OF THE SEX-RATIOS OF THE OFFSPRING OF PURE AND CROSS MATINGS.

Matings.		Sex Ratio.	$\frac{\text{Difference}}{\text{P.E. of Difference}}$
Italian ♂	Argentine ♀	105.72 ± .46	
Italian ♂	Italian ♀	100.77 ± .20	
Difference		4.95 ± .50	9.9
Italian ♂	Argentine ♀	105.72 ± .46	
Argentine ♂	Argentine ♀	103.26 ± .34	
Difference		2.46 ± .57	4.3
Spanish ♂	Argentine ♀	106.69 ± .74	
Spanish ♂	Spanish ♀	105.55 ± .36	
Difference		1.14 ± .82	1.4
Spanish ♂	Argentine ♀	106.69 ± .74	
Argentine ♂	Argentine ♀	103.26 ± .34	
Difference		3.43 ± .81	4.2

From this table it appears that the excess of male births in the cross matings as compared with the pure is in general large in proportion to its probable error. In only one out of the four

possible comparison cases is the difference less than four times its probable error. In that case (Spanish-Argentine and Spanish-Spanish) the difference is 1.4 times its probable error, and could not, taken by itself, be considered significant. Taking into account, however, the facts that (*a*) the difference is of the same sense as the other differences in the table and (*b*) that it is larger than its probable error the general conclusion reached from the other figures is not vitiated. This conclusion is that within the limits of the present material *there is evidence of the significantly greater proportionate production of males in the offspring from matings involving different racial stocks than in the offspring from matings in which both parents belong to the same racial stock.*

Discussion.

The data which are set forth in the tables given above appear to lead clearly to the conclusion which has been drawn from them. This conclusion, however, is merely a statement of fact. In interpreting it it remains to consider two points. The first of these is as to whether there are limitations or fallacies in the data themselves which invalidate the conclusion to which they appear to lead. The second is as to what is the meaning of the facts implied by this conclusion supposing it to be true. One cannot be too cautious in drawing conclusions from human vital statistics of whatever kind. Vital statistics notoriously abound in pitfalls. In a critical examination of the data with a view to possible criticism and interpretation the following points suggest themselves:

1. That the material is not sufficiently extensive. It might conceivably be maintained that if a larger number of births were to be dealt with they would show a different result. For two reasons such a consideration appears to have little weight. In the first place the number of births included in the statistics is extremely large as measured by biological standards. The statistics include upwards of 200,000 births. In the second place the probable errors of the sex-ratios indicate how literally enormous are the combined odds against such a consistent system of differences as that shown in Table III., arising fortuitously. In this connection it may be said that the work was begun in the

first instance with the statistics for three years (1903, 1904 and 1905) only. The figures for these years led to exactly the results which have been shown above. The figures for the seven previous years were then taken into the calculation to see whether they would confirm or reverse these results. That they confirm them is clear.

2. That the inclusion of living births only in the statistics influences the result. That statistics of sex should theoretically include still-born as well as those born living is obvious. The still-born would have been included in the tables of this paper had it not been for the fact that the original material was tabulated in such way as to render it impossible to include them. A little consideration shows, however, that the absence of still-born does not sensibly affect the conclusion drawn from the present statistics. It is a well-known fact that among still-born children the proportion of males to females is very much greater than among living born. It does not seem necessary to cite evidence of this fact; all large collections of birth statistics show it. Pains have been taken to make sure that the records of still-born in Buénos Ayres form no exception to the general rule.

Now there are three possibilities respecting the distribution of still-born young among the offspring of the cross and pure matings discussed in this paper. These are:

(*a*) That still-births are distributed *pro rata* among cross and pure matings. This is the most probable supposition. It would be expected on general grounds that in the long run there would be substantially the same number of still-births among a given total number of births whether this total originated from cross or pure matings.

(*b*) That a relatively larger number of still-births originate from pure than from cross matings.

(*c*) That a relatively large number of still-births originate from cross matings than from pure.

It being a fact that still-births show a high sex-ratio it is evident that a distribution of such births in accordance with (*b*) could alone tend to reverse the conclusion reached from statistics which leave these births out of account. In case they were distributed as in (*a*) or (*c*) their inclusion would simply make

more pronounced the results found in their absence. It appears highly probable on general grounds that if still-births are not proportionately distributed among cross and pure matings there is somewhat more likely to be an excess of such births arising from cross (*i. e.*, according to (*c*)) than from pure matings (*i. e.*, according to (*b*)). It is hardly conceivable that there could be a steady tendency for a sensibly greater number of still-births to occur when both parents are of the same nationality than when they are of different nationalities. If this be granted then it must also be granted that the non-inclusion of still-births in the present statistics cannot be adduced as an explanation of the observed preponderence of males in the offspring of cross matings.

3. That a different age distribution of the parents in cross as compared with pure matings may account for the observed preponderance of male births from such matings. In a population such as that here dealt with it is undoubtedly true that the males in the cross matings (being for the most part probably immigrants) are on the average somewhat older than those in the pure matings. It might conceivably be contended that this greater average age of the male parent was the cause of the excessive production of male offspring in the cross matings. To make such a contention, however, would simply be to affirm belief in Sadler's "law"[1] or some variant of it which holds that the relative age of the parents is causally related to the sex-ratio of the offspring. In regard to this matter it need only be said that Sadler's theory has been abandoned by all recent students (both from the biological and from the demographic side) of the problem of sex simply for the reason that nothing remotely approaching conclusive evidence has ever been brought forward in its support.

4. That the individuals in the cross mating are exposed to environmental influences different on the average from those acting on the individuals in pure matings and that the differences in the sex-ratios of the offspring of these two groups are the result of these environmental differences. This possible explanation obviously needs careful consideration. So far as broad environ-

[1] *Cf.* Geddes and Thomson, "The Evolution of Sex" or any of the standard works on vital statistics for an account of this law.

mental factors such as climate are concerned there can be no differential effect on the sex-ratio for the two groups since all the statistics are derived from the population of a single city. In a general sense all the individuals live in the same environment. But there is a possibility of a difference between the different groups in regard to the complex of environmental factors which are collectively implied in "social status." It is conceivable that on the average the Italian-Argentine families are of different social status than Italian-Italian or Argentine-Argentine families in the same city. Differences in social status imply differences in nutrition, in housing and in other physical conditions of existence. Some one or all of these things might conceivably be held to affect the sex-ratio in the manner observed. In considering this point it needs to be held clearly in mind that there are two distinct questions involved. These are: (*a*) Is there any conclusive evidence that there does exist as a matter of fact any uniform average difference in the social status of individuals in cross as compared with pure matings? And (*b*) granting that such an average difference does exist what evidence is there that it would produce the observed effect on the sex-ratio? To the first of these questions it is difficult to get any answer. Careful study of all the available demographic statistics of Buénos Ayres has failed to yield any conclusive evidence on the point. The probability appears to be, however, that if any difference at all exists in the social status of the two groups it is in the long run (or on the average) not marked in degree. Further it appears probable that whatever difference does exist is in the direction of a lower social status in the case of the cross matings.

Regarding the influence of such a difference (if it exists) on the sex-ratio it seems probable that it would have very little or no effect. Punnett[1] has recently made a very careful study of just this point for certain elements of the population of London. He finds that in the classes of lower social status more females than males are born, and *vice versa*, but concludes in general that parental nutrition has no sensible influence on the sex-ratio. Morgan[2] reviews the literature on the subject and reaches the fol-

[1] Punnett, R. C., "On Nutrition and Sex Determination in Man," *Proc. Camb. Phil. Soc.*, Vol. 12, 1903.

[2] Morgan, T. H., "Experimental Zoölogy," New York, 1907, pp. xii + 454.

lowing conclusion (p. 385): "If nutrition were really a factor of any importance in sex determination, it is surprising to find so little difference under apparently very favorable and unfavorable conditions. It seems much more probable that if the nutrition affects in any way the proportion of the sexes, it does so indirectly by elimination, and not by determining either the sex of the embryo or of the egg." Further on Morgan says in discussing Geddes and Thomson's theory of sex (p. 388): "If, on the other hand, the determination of sex is supposed to be due to the nourishment of the embryo, the best ascertained facts, both experimental and statistical, are opposed to the hypothesis." Taking all these points into consideration it seems very doubtful, to say the least, if the observed excess of males in the cross matings has its explanation either in whole or in part in differences in the environmental complex implied by "social status." However, in the absence of more complete and definite statistical data regarding the point one cannot be dogmatic in asserting such a conclusion.

If none of the suggested factors can reasonably be held to afford an explanation of the facts regarding the sex-ratio shown by the present statistics how are these facts to be interpreted? All that can safely be asserted is that the present statistics, within their limits, show clearly that there is a definite relation between the character of the mating and the magnitude of the sex-ratio. Is this a *post hoc* or a *propter hoc* relation? The data themselves do not *conclusively* demonstrate which it is. Nor does it seem probable that statistics of human births alone can ever settle this question. It is one which demands experimental analysis. The chief difficulty involved in maintaining that there is a causal relation between the character of the mating and the sex-ratio lies in the lack of knowledge as to what could be the physiological mechanism by which the causation was effected. In a way the phenomenon appears somewhat analogous to the well-known phenomenon of xenia observed in plant breeding, differing in that here the character influenced is sex rather than some purely morphological feature of the organism.

In conclusion it should be said that the data presented in this paper are not put forth as in any way final or conclusive. They

require confirmation from other sources and *experimental* analysis. Within their limits, they lead to a definite and significant conclusion as to fact. In so far they contribute to the discussion of the general problem of determination of sex.

Summary.

Statistics of over 200,000 human births extending over a period of 10 years in the city of Buénos Ayres show that the proportion of males to females is significantly greater when the parents are of different racial stocks than when they are of the same. In the data are involved three racial stocks in pure and cross matings. The preponderance of males in the offspring of cross matings appears not to be capable of explanation as the result of environmental or demographic influences. Experimental investigations are necessary in order to reach adequate explanations of such statistical facts regarding sex ratios as are set forth in this paper.

Biological Laboratory,
Maine Experiment Station, Orono, Maine.

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BIOLOGICAL BULLETIN

A SIGNIFICANT CASE OF HERMAPHRODITISM IN FISH.[1]

H. H. NEWMAN.

The subject of hermaphroditism in fish has received the attention of only a few workers. Our principal information is derived from the work of Stephan ('01). This author describes for certain species of fish a complete and simultaneous hermaphroditism, ripe ova and spermatozoa appearing in the same individual at the same time; for other species a protandric hermaphroditism, the individuals while young being males and later in life becoming females; for still others, a precocious appearance of sexuality in the males and a tardy appearance of the latter in the females of species still considered as unisexual.

The condition last mentioned is interpreted by Roule ('02), on the basis of rather doubtful evidence, as a true case of protandric hermaphroditism. He measured large numbers of sexually mature individuals belonging to several species of Cyprinidæ, and found that all of the individuals of small size were males and all those of large size were females. Hence, according to Roule, all individuals are males when young and females when older. The only alternative interpretation of the facts presented seems to be that these species exhibit strict unisexuality of all individuals, with dwarfing of the males and precocious appearance of male sexuality. Roule points out, however, that, on this basis, one would expect to find among the smaller individuals young females with immature sex glands, and that there should be at least as many of the latter as there are adult females. But none such were found by him.

[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 94.

Roule's paper, being simply a preliminary statement, is too inadequate to furnish the basis for a detailed discussion, yet it might be well to point out that the Pœciliidæ, a family rather closely related to the Cyprinidæ with which Roule worked and about which there can be no suspicion of normal hermaphroditism, exhibit conditions closely parallel to those cited by Roule.

Let us take, for example, the state of affairs in *Fundulus majalis*. Here the mature males are, on the average, considerably smaller than the mature females; yet the largest of the males often surpass in size the smaller sized females. Again, the very smallest sexually mature individuals are always males and the very largest are always females. The males also mature distinctly earlier in the season than do the females. All of these facts attest the precocity and dwarfing of the males.

In view of the fact, however, that in *F. majalis* there is a very pronounced sexual dimorphism that begins to make itself apparent in very young and immature fish, it becomes certain that all individuals are unisexual throughout life. The individual whose discovery gave occasion for this paper, is the only exception to this rule that has come under the observation of the writer although he has examined thousands of specimens of this and allied species during the last three years.

In order that the reader may more readily understand the account of this rather remarkable case of hermaphroditism it seems necessary to recapitulate certain facts concerning the sexual dimorphism and spawning behavior of *Fundulus majalis*, a subject treated extensively in former papers (Newman, '07 and '08).

In *F. majalis* the sexes differ in the following particulars:

1. The females are larger, on the average, than the males.

2. The body color pattern of the two sexes is entirely different; that of the male consisting of distinct transverse bands running from back of the head to near the base of the caudal fin (see Fig. 1); that of the female, on the other hand, consisting essentially of well-marked longitudinal stripes, perfect anteriorly and merging posteriorly into a few cross bands like those of the male (see Fig. 4).

3. The cross-banded pattern is the primitive one for the family as well as for the species, and all young fish of both sexes start out with this pattern. The males retain this juvenile pattern, in a somewhat strengthened form, throughout adult life. In the females, however, the primitive cross-banded pattern is gradually transformed into one characterized by longitudinal stripes, in the following manner. The cross bands, beginning with the anterior ones, show thickenings in two places. The parts of the bars between these thickened regions thin out and disappear, leaving

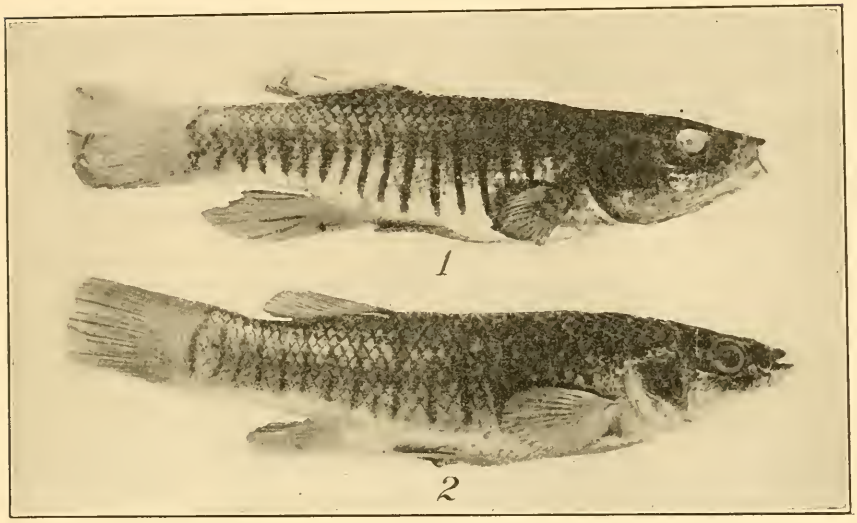


FIG. 1. Photograph of preserved specimen of male *Fundulus majalis* from the right side (slightly reduced). This specimen shows the dark spawning coloration about head and back, the large size of dorsal and anal fins, the typical cross-banded pattern. The characteristic male marking on the dorsal fin, however, shows only faintly.

FIG. 2. Photograph of the hermaphrodite specimen of *F. majalis*, taken with the same exposure as Fig. 1. Note that the general body coloration is almost that of the male, but that the cross-banded pattern is weaker. This pattern, however, was much stronger in life. In other respects it resembles the female (Fig. 4).

the thickened regions arranged in two rows. These then fuse longitudinally into two or more stripes. This process is described fully and figured in a former paper (Newman, '07).

4. The male is characterized by the presence of a very prominent dark spot or series of spots, surrounded by a light area situated on the posterior rays of the dorsal fin. The photograph

(Fig. 1) does not do this character justice. A far better idea of the prominence of this sexual marking can be obtained from an examination of the illustration in the paper just referred to.

5. The dorsal and anal fins of the male are much larger and stronger than those of the female and are used as clasping organs in spawning.

6. These fins and all parts of the body of the male that come into intimate contact with that of the female in spawning and courtship are covered with small finger-like papillæ that lend to these parts a decided roughness and undoubtedly assist the male in holding the female securely. These organs have elsewhere been designated "contact organs."

7. During the sexual climax the whole body of the male is suffused with dark pigment, some specimens showing an almost inky blackness on head, cheeks and back. The female, however, retains her normal pale olivaceous tint, or in many cases becomes distinctly paler than during the vegetative season.

8. The flesh of the female, during the height of the spawning season, becomes softer than usual and the abdomen is greatly distended with ripe ova.

9. The behavior of the males, during the spawning season, is sharply contrasted with that of the females. The former are spirited and pugnacious, and frequently follow the females about in order to spawn with them. Actual spawning, however, was observed only occasionally in *F. majalis*, but it is essentially like that of *F. heteroclitus*, which was observed hundreds of times. The behavior of the female is characteristically coy.

These and a few minor differences between the sexes will serve to render intelligible the account of the individual now to be described.

Description of the Hermaphrodite Specimen.

The fish herein described was discovered by merest chance during the progress of some breeding experiments at the Woods Hole laboratories.

On July 3, 1907, needing a male *Fundulus majalis*, I rather hurriedly dipped out of the aquarium what I took to be a large, but decidedly pale, male. Wishing to perform an experiment

with the milt of just such a male as this seemed to be, I attempted, without further examination, to strip milt from the specimen. Instead of milt a stream of eggs issued from the short genital tube at the base of the anal fin. I knew, of course, that fish frequently eat eggs and pass them undigested through the digestive tract, but such eggs are always dead and opaque, while these eggs were normally transparent. Surprised at the extrusion of eggs from an individual supposedly male I proceeded to make a careful examination. This revealed the fact that the fish was male only in one respect. It showed the cross-banded body pattern of the male very distinctly. It lacked, however, the characteristic spot on the dorsal fin, the large size of dorsal and anal fins, contact organs, and intensified pigment of the typical male ; while it possessed the distended abdomen soft flesh, lighter ground color, small fins, and external oviducal tube of the spawning female. Yet I had never before seen an adult or even a juvenile female without longitudinal stripes distinctly indicated.

The specimen seemed sufficiently unusual to deserve a separate aquarium, where it was well fed and relieved of its burden of eggs several times during the ensuing fortnight. These eggs showed a rather low degree of fertility, although at least ten per cent. developed in each case.

After about a week a typically marked female of about the same size was introduced into the special aquarium for the sake of comparison, and both normal and abnormal specimens were treated alike. For nearly two weeks the two fish behaved alike, but after that time the cross-banded fish began to lose its quiet passive behavior and to assume a decidedly overbearing attitude toward its companion. Several of my fellow investigators called my attention to this curious behavior, which might well be termed "bossy." Accompanying this change in behavior were several morphological changes. The body became slimmer, as would be expected since the eggs had practically all been extruded, the flesh became harder, and dark pigment was laid down all over the body. The latter was most noticeable on head and cheeks which had become decidedly dusky, a change very characteristic of males entering upon the period of high sexual tone. The cross bands become darker and more distinct and a faint wash of orange tint appeared on the anal fin, a distinctly male character.

During the last week of July the fish was kept under close observation. On several occasions it showed a type of behavior distinctly male-like. It followed the female about and repeatedly made movements that seemed to indicate a weak attempt at spawning. Of this I could not be positive, but in other respects the behavior was that of a courting male. It would have surprised me greatly had there been an exhibition of actual spawning, for, as has been said, *F. majalis* seldom spawns in captivity.

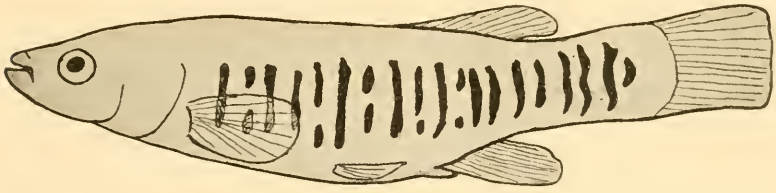
On the last day of July the writer was compelled to leave the laboratories and the fish was killed and carefully preserved in formalin.

An examination of the formalin-preserved sex gland revealed the fact that it was a composite gland, containing about five per cent. of testicular tissue, slightly immature, and imbedded in a mass of immature and stale ovarian tissue. The testicular tissue occurred in minute lumps, principally near the posterior end of the gland. Although distinctly testicular in structure, these small masses showed a less typical structure than that of normal testis, being less compact and interspersed with connective tissue. The color, size and general appearance of the whole gland was that of a preserved testis, there being no yellow color present as is the case in normal ovaries after the close of the spawning season.

A further close examination of the body pattern showed that the cross banding on right and left sides was not equally perfect, that of the right side showing the character in as perfect a form, if less distinct, as in a typical male, that of the left being partially broken into shorter bars, the beginning of a tendency on the part of the cross-banded pattern to break up into the rows of spots that furnish the material for longitudinal stripes.

The photograph of the specimen taken from the right side shows the pattern far less distinctly than in life on account of the fact that scales and skin, rendered opaque by the preservative, obscure the underlying markings. In other respects the illustration (Fig. 2) is a faithful representation of the conditions. Photographs of the left side failed entirely to bring out the points desired, so it was necessary to insert a camera drawing as the best substitute. This drawing (Fig. 3), showing the less perfect

cross banding, when compared with the average condition seen in an adult female (Fig. 4), will show a striking contrast. It will be noted that the hermaphrodite exhibits a somewhat juvenile figure in that the head is shorter, the body broader and the tail less tapering. These points might not be patent to one not very familiar with the species.



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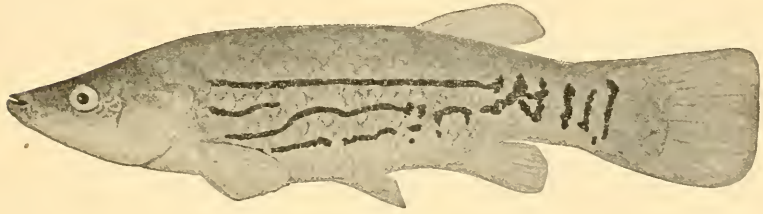

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FIG. 3. Outline camera drawing of the hermaphrodite from the left side, to show the rather broken character of the cross-banded pattern.

FIG. 4. Wash drawing, showing a typical female *F. majalis*. Many specimens show a far more complete transformation of the juvenile cross-banded pattern into the series of longitudinal stripes but few females of this size show a less advanced condition. Note the long head, comparatively small dorsal and anal fins, and comparatively light ground color of head and back.

General Considerations.

1. The extreme rarity of hermaphroditism in fish normally unisexual makes this case worthy of note, especially as the sexes are so well differentiated in form, color pattern, and behavior. No other case comparable with this is on record. There seems, in fact to be only one case of abnormal hermaphroditism in fish in the available literature. This is a brief description by Southwell ('02), of a hermaphrodite gland taken from a smoked her-

ring. The only point of interest for this discussion, since herrings seem to show no sexual dimorphism, is that the testicular tissue was located posterior to the ovarian tissue and overlapped the latter somewhat. This condition of the sex glands reminds one of the composite gland described in the preceding paragraphs.

2. Some light is thrown on the influence of the sexual secretions upon the secondary sexual characters. In this case the presence of a comparatively minute amount of imperfect testicular tissue has had the negative effect of inhibiting, in an individual predominantly female, the transformation of the juvenile into the female color pattern ; and the positive effect of producing in this individual, at the expiration of the season's period of egg production, an approximation of male coloration and behavior.

3. In all cases of serial hermaphroditism described in available literature the hermaphroditism is protandric and in successive seasons. Here the sequence was distinctly protogynic and the changes occurred within a period of less than a month. The condition is decidedly anomalous.

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THE HOMING OF THE MUD-DAUBER.

C. H. TURNER.

Introduction.

In my paper on "The Homing of Ants"[1] there is recorded evidence that ants find the way home neither by a homing instinct nor by reflex action nor merely by kinesthetic responses; but by utilizing landmarks. In this paper I propose to record experimental evidence that the same is true of the common mud-dauber wasps.

It has long been recorded by keen observers that both the social and the solitary wasps, on leaving their nests for the first time, carefully examine the surroundings before flying away. It is also stated by some that any alteration in the immediate surroundings of the nest will render it difficult, or even impossible, for the wasp to find its way back again.

Mr. and Mrs. Peckham, who have devoted much time to the study of both the social and the solitary wasps, say:[2] "If they were furnished with an innate sense of direction they would not need to make a study of the locality of the nest in order to find the way back, but if they were without this sense it would be only common prudence to take a good account of their bearings before going far afield. . . . In reading much of the popular natural history of the day one might suppose that the insects seen flying about on a summer's day were a part of some great throng which is ever moving onward, those that are here today being replaced by a new set tomorrow. Except during certain seasons the exact opposite is true. The flying things about us abide in the same locality and are the inhabitants of a fairly restricted area. The garden in which we worked was, to a large extent, the home of a limited number of certain species of wasps that had resided there from birth or, having found the place accidentally, had settled

[1] *Jour. of Comp. Neur. and Psy.*, Vol. XVII., pp. 367–434, Pl. II.–IV.

[2] G. W. and E. G. Peckham, "On the Instincts and Habits of the Solitary Wasps," Madison, Wis., 1898, pp. 212, 213, 215.

there permanently. . . . After days passed in flying about the garden — going up Bean Street and down Onion Avenue, time and again — one would think that any formal study of the precise locality of a nest might be omitted, but it was not so with our wasps. They made repeated and detailed studies of the surroundings of their nests. Moreover, when their prey was laid down for a moment on the way home, they felt the necessity of noting the place carefully before leaving it. . . . If the examination of the objects about the nest makes no impression upon the wasp, or if it is not remembered, she ought not to be inconvenienced nor thrown off her track when weeds and stones are removed and the surface of the ground is smoothed over; but this is just what happens. *Aporus fasciatus* entirely lost her way when we broke off the leaf that covered her nest, but found it, without trouble, when the missing object was replaced. All the species of *Cerceris* were extremely annoyed if we placed any new object near their nesting-places. Our *Ammophila* refused to make use of her burrow after we had drawn some deep lines in the dust before it. The same annoyance is exhibited when there is any change made near the spot upon which the prey of the wasp, whatever it may be, is placed. We learned from experience how important it was not to disarrange the grass or plants on such occasions."

All this was written before Bethe[1] had restated, with emphasis, his theory that bees (the morphological and physiological kinship of which to wasps leads one to expect them to be psychologically similar) are guided home by an unknown force; and before Pieron[2] had asserted that ants are led home by a reflex kinesthetic sense. This being the state of affairs a crucial experiment seemed to be needed. The mud-dauber (*Sceliphron*, Klug = *Pelopœus*, Latr.) was selected, partly because its habits rendered it comparatively easy to obtain material and partly because, so far as I know, no such experiments have been performed upon it.

[1] A. Bethe, "Die Heimkehrfähigkeit der Ameisen u. Bienen zum Theil nach neuen Versuchen," *Biol. Centrlbl.*, 2 Bd. (1902), no. 7, pp. 193–215; no. 8, pp. 234–238.

[2] H. Pieron, "Du rôle sense musculaire dans l'orientation des fourmis," *Bull. Inst. Gen. Psy. Paris*, T. 4 (1904), pp. 168–187.

Preliminary Observations.

These preliminary observations were made in a laboratory the walls of which were ceiled with tongue-and-grooved pine boards. These boards were arranged vertically. Two of the walls were supplied with windows and two were not. Near the top of each of these walls mud-daubers constructed nests. Some of these nests were in dark places and some were in light places. I noticed that the wasp never flew directly to the nest, but that it would alight on a certain crack. After ascending, afoot, this crack, until it had reached the height of the nest, it would turn and walk to it. The same wasp always alighted on the same crack and at about the same distance from the floor. This led me to suppose that wasps used the cracks as landmarks. In this room a certain window was lowered from the top, through which opening the wasps came and departed. In another room, in which similar experiments were conducted, the window was raised from the bottom. Wasps frequented this room as much as they did the other. Evidently wasps can learn the way into a room by either a high or a low opening.

The Environment of the Experiments.

This series of experiments was performed in a laboratory thirty-seven and a half feet long, twenty-five feet wide and twelve feet

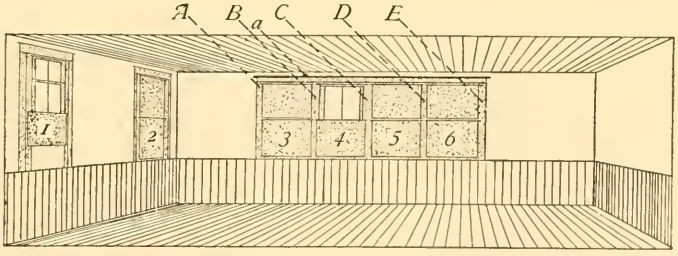

Fig. 1. This is a diagram of the room arranged for experiment one. 1–6, windows; *A–E*, upright facings of windows; *a*, location of the nest. The wood-work of the windows is shaded with broken lines, the window-shades with dots. The boards in the floor, ceiling and dado are drawn twice as wide as they were.

high (Fig. 1) which was situated in the third story of a large brick building. The ceiling was covered with tongue-and-grooved pine boards and a four-foot dado of pine ceiling extended around

the lower third of the walls of the room. Excepting the space occupied by windows, doors and dado, the walls were plastered in the rough. In the west wall there were two windows (Fig. 1, 1 and 2); in the north, four (Fig. 1, 3 to 6). The windows of the north wall were close together, being separated by wooden partitions only one foot wide (Fig. 1, *B*, *C*, *D*). Across the top of each window there was a piece of three inch moulding. On the north wall this moulding extended continuously across the four windows. There were two green blinds to each window, one to each sash. These blinds were not quite opaque. The ceiling was painted green; the walls, including dado, doors and window-facings, cream color.

In order to have only one entrance for the wasps, the lower sash of window number one was raised half way. All other windows were closed. This condition was maintained throughout the entire series of experiments. To furnish definite light relations for the beginning of the experiment, the lower shade of window number one was raised about half way and the top shades of windows one and three raised as far as possible. All the other shades were down. After these conditions had been maintained for nearly two weeks a mud-dauber began the construction of a nest on the moulding above window number three at a point about six inches from upright *B* (Fig. 1, *a*).

When first discovered the wasp had completed nearly half of one cell; hence it had already made several trips back and forth through window number one. I watched the wasp make several trips, and each time it behaved as follows. On entering the room it would fly obliquely upwards to the upper third of upright *B*. Then it would fly vertically upwards almost to the ceiling, thence it would fly leftward to the nest. The line of flight from the entrance to upright *B* was perceptibly curved, the convexity being towards the east. The flight from the entrance through window number one to the nest consumed about half a minute. In departing, the wasp flew downwards in a curve from the nest to the upper portion of the opening through which it had entered the room. After watching the wasp make several trips in practically the same manner, the following experiments were performed:

EXPERIMENT 1.

The lower shade of window number one was raised half way and the top shade as far as it would go. While the wasp was out of the room, all the blinds of windows number two to six were closed except the upper shade of window number four, which was raised as far as possible (Fig. 1).

The wasp on entering through window number one flew obliquely upwards across the beam of light from window number four to the upper third of upright *C*. (This line of flight was convex towards the east.) It then flew vertically upwards almost to the ceiling then leftward about a foot (this is a little more than the distance of the nest from upright *B*) and examined carefully the moulding. Not finding the nest, it began flying first to the right and then to the left in constantly elongating ellipses with very short minor axes. All this time it was carefully examining the moulding. Occasionally the mud-dauber would fly downward into the beam of light and then resume its search. In its lateral flights the wasp sometimes flew as far to the east as upright *D* and to the west almost as far as upright *B*. At the end of three minutes it had not found the nest, although under former conditions of illumination it required only half a minute to fly from window number one to the nest.

While the wasp was still searching for the nest, the top shade of window number four was lowered and the corresponding shade of window number three raised as far as possible. This reproduced the conditions under which the wasp had originally worked.

Almost immediately the wasp found the nest!

EXPERIMENT 2.

The lower shade of window number one was raised half way and the top shade as far as possible. While the wasp was out of doors, all the shades of windows number two to six were lowered except the top shade of window number five, which was raised as high as possible.

On entering, the wasp flew in a fairly direct line towards the nest. When about one third of the way across the room, it returned almost to window number one and described a circle of about a foot in diameter. It then flew to the middle of the upper

shade of window number four. Thence it flew upwards almost to the ceiling and then leftward to the nest.

Experiment 3.

The lower shade of window number one was raised half way and the upper shade as far as possible. All of the shades of windows number two to six were lowered except the top shade of window number three, which was raised as far as possible. This reproduced the conditions under which the wasp had worked originally.

On entering the room the wasp flew obliquely upwards to the upper third of upright *B*. Then it flew vertically upwards almost to the ceiling. Thence it flew leftward to the nest. The line of flight from the entrance to upright *B* was perceptibly curved, the convexity extending towards the east. The total flight from the entrance to the nest consumed about half a minute. The shades were maintained in the above position until the mud-dauber had made three trips. Each was made in practically the same manner.

Experiment 4.

The same conditions as in experiment one.

The behavior was practically the same as in experiment one. In this experiment, however, the shades were maintained in the same position until the wasp had found the nest, which required nearly five minutes.

The wasp was allowed to make two trips. Its behavior on the second trip resembled that on the first ; but it required only three minutes to pass from the entrance to the nest.

Experiment 5.

The same conditions as in experiment three.

The wasp behaved the same as in experiment three.

Experiment 6.

The lower shade of window number one was raised half way and the top shade as high as possible. All of the shades of windows two to six were lowered, except the top shade of window number two, which was raised as far as possible.

The wasp on entering the room described a small circle then

flew obliquely upwards to a point almost to the ceiling, but a little to the west of upright *A*. It then flew alternately leftward and rightward until the nest was found, which consumed about one minute.

The wasp was allowed to make two trips. Its behavior on the second trip was similiar to that on the first, and about the same amount of time was consumed in passing from the entrance to the nest.

Experiment 7.

The conditions were the same as in experiments three and five.

The wasp was permitted to make two trips. It behaved the same as it did in experiments three and five.

Experiment 8.

The lower shade of window number one was raised half way and the top shade as high as possible. All the shades of windows two to four were lowered.

On entering the room the wasp described several small circles. It then flew first to about the middle of the upper sash of window number three, then to window number two, then to window number five. Finally, after much searching, the nest was found.

The shades were retained in the above condition until the wasp had made two trips. On the second trip it went first to upright *C* and then, after a short search, it found the nest.

Experiment 9.

The same conditions as in experiments three, five and seven.

The behavior was the same as in experiments three, five and seven. Two trips were made.

Experiment 10.

At first the conditions were the same as in experiment eight. While the wasp was on the nest, the top shade of window number one was lowered.

On leaving the nest the wasp flew away through the window in its usual way. The lowering of the top shade of window *A* did not change its behavior.

Experiment 11.

The lower shade of window number one was raised half way and the top shade lowered. While the wasp was out of doors, all the shades of windows two to six were lowered except the top shade of window three, which was raised as high as possible.

The wasp behaved the same as in experiments three, five, seven and nine.

Experiment 12.

The lower shade of window number one was raised half way. While the wasp was out of doors, all of the other shades were lowered.

The wasp behaved the same as in experiment eight.

Experiment 13.

The lower shade of window number one was raised about half way. While the wasp was out of doors, all the shades of windows two to six were lowered except the lower shade of window number three, which was raised as far as possible.

At first the wasp searched carefully the upper portion of the wall to the west of the upright *A*. Then, as a result of extending its search to the east, the nest was discovered.

This experiment was repeated with the same results.

Experiment 14.

The same conditions as in experiments three, five, seven, nine and eleven.

The wasp behaved the same as it did in experiments three, five, seven, nine and eleven.

Experiment 15.

The same conditions as in experiment one.

The wasp behaved as in experiment one. It took about one minute to find the nest. The wasp was allowed to make two trips.

Experiment 16.

The same conditions as in experiment one.

After the shades had been maintained in this position for about two days, the wasp on entering the room flew obliquely upwards

to near the top of upright *C* and then obliquely leftward and upwards to the nest. The trip from window number one to the nest consumed much less than a minute.

Experiment 17.[1]

The same conditions as in experiment three. This experiment was performed immediately after the close of experiment sixteen.

The wasp on entering the room flew obliquely upwards, across window number four, to the upper third of upright *C*; then obliquely leftward and upwards, across windows number four and three, to a little beyond the upright *A*. It then searched about until the nest was found.

The shades were left in this condition from 10:45 A. M. July 19 to 9 A. M. July 20, at which time the wasp occasionally visited the nest. The wasp on entering the room, flew obliquely upwards, across window number three, to the upper third of upright *B*; then leftward, across window number three, to a little beyond upright *A*, then obliquely rightward and upwards to the nest. The conditions in this experiment and in experiments three, five, seven, nine and eleven are identical, yet the behavior in this case is quite unlike what it was in those. Evidently prolonged exposure to the conditions described in experiment sixteen has modified the behavior of the wasp. It had lost (forgotten) its old response to the conditions described in experiment three and been forced to acquire a new response.

Conclusions.

From these experiments it is evident that, in finding its way back to its nest, the mud-dauber is guided neither by what is known as a homing instinct nor by what Pieron has called a kinesthetic reflex; for if either assumption were true, a manipulation of the light should not have altered the wasp's behavior.

Evidently light plays a prominent rôle in the homing of wasps, yet the behavior of the mud-dauber is not a phototropism; for in

[1] This series of experiments was begun on the morning of July 17, 1908, and ended on the morning of July 20, 1908. Experiments one to fifteen inclusive were performed the first day and in the order mentioned. The intervals between the experiments were only sufficiently long to permit the necessary adjustments to be made. Between experiments fifteen and sixteen there was an intermission of almost two days; between experiments sixteen and seventeen, an intermission of five minutes.

no case did the wasp so orient itself as to have the major axis of its body parallel to the rays of light. Furthermore in hunting for the nest, the wasp crossed the light sometimes in one direction and sometimes in another. In yet other cases the wasp would zigzag across the light.

Neither is the wasp's behavior merely a reflex response either to brightness or to the direction of the rays of light; for if that were the case, in experiment six, when all the shades of windows number two to four were lowered except the top shade of window number two, the wasp should have flown, not to the wall to the west of window number three, but to window number two. Likewise in experiment eight, when all the shades of windows number two to six were lowered and the only bright light entering the room was that which came through the upper and lower portions of window number one, if the wasp were guided merely by light acting reflexly, then it should not have been able to find the nest at all. Furthermore, if the wasp's behavior is merely a reflex response to light, there is no reason why it should have entered the room at all, for the open portion of the window was certainly not so bright as the window-panes from which the light was reflected. We say nothing about the bright sunshine out of doors!

But brightness is not the only factor which influences the movements of this wasp; else, when all the shades of windows number two to six were lowered, it would have been impossible for it to rediscover the nest. This series of experiments warrants the induction that, in the wasp's memory, that nest is located in a certain direction and at about a definite distance from a bright patch which is situated at a known elevation in a peculiar environment.

The above statement predicates to wasps memory and an awareness of space relations. As to the existence of memory these experiments furnish unequivocal evidence. This harmonizes with the views of Forel and the Peckhams. In "The Homing of Ants" are recorded proofs that ants have an awareness of space relations, and, since wasps are near kin to ants, it is probable that they, too, have an awareness of space relations. This series of experiments furnishes evidence to support this view. In

almost every experiment of this series the lower shade of window number one was raised half way and the top curtain all of the way. This was done in order to have the departing wasp confronted, on each trip, by an upper and a lower bright patch. Were the wasp responding to a bright patch merely and not to a bright patch in a definite place, then the wasp should have flown to the upper bright patch just about as often as it did to the lower. The wasp always flew directly from the nest to the opening in window number one! There was but one exception to this statement. On one occasion I was standing on a ladder watching the wasp construct the nest. I was within two feet of the nest. On that occasion the wasp, on departing, circled about once or twice and then returned to the nest and from there flew to the exit.

In brief, these experiments warrant the conclusion that the flying mud-dauber, like the creeping ant, is guided by certain landmarks, and that light plays a prominent rôle in furnishing such landmarks.

HAINES NORMAL SCHOOL,
AUGUSTA, GA., July 25, 1908.

EXTRUSION OF THE WINTER EGG CAPSULE IN PLANARIA SIMPLISSISSIMA.[1]

FLOYD E. CHIDESTER,

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About the middle of October, 1907, I chanced to note that some of the *Planaria simplissisima* in one of my aquaria had developed egg capsules.

I immediately transferred a number of flatworms, including those that had already formed capsules, and a few that had not as yet developed them, to a small aquarium on my desk. While examining one of the planarians and its capsule under the microscope, I saw the movements connected with the extrusion of the capsule.

When first observed, the capsule lay lengthwise of the body in the position indicated in Fig. 1.

Presently the capsule was turned by the movement of the body of the worm until it occupied a position as indicated in Fig. 2,

FIG. 1.

FIG. 2.

FIG. 3.

then slowly came to the position indicated by Fig. 3, that is, at right angles to the longitudinal axis of the body.

This change in position necessarily increased the size of the cavity in which the capsule lay, lacerating the tissue and permitting easy egress.

[1] Contributions from the Biological Laboratory, Clark University.

The planarian then moved slowly forward, the capsule passing along through its body and out at the dorsal caudad region. The rotation of the capsule in the body of the animal apparently aided in breaking the wall of the cavity in which the capsule lay, for there seemed to be no difficulty in the passage through the posterior portion of the body and out near the tail (Fig. 4).

The entire process of extrusion occupied only about thirty minutes.

The next day the wound where the capsule had originally lain was partly closed by contraction of the surrounding tissue, and the wound in the tail region made at the escape of the capsule was obliterated almost entirely.

I was interested to see if the same planarian forms more than

FIG. 4.

FIG. 5.

one egg capsule, so isolated the animal I had been observing, and examined it several times each day.

In a week complete regeneration was effected. At the end of twenty days from the time I had seen the first capsule, another appeared, evidently formed during the night, and I had the good fortune to observe its extrusion also. The movements were the same as before observed, except that the capsule was extruded on the right side of the body this time.

Other planarians bearing capsules were observed, and in all cases the capsule was rotated immediately before the extrusion

The individual described previously, at the end of about three weeks more, extruded a third capsule.

Kept in a small vial on my desk for two months after this extrusion, the planarian formed no more capsules.

The winter egg capsule of *Planaria simplississima* is a dark brown, elongated object, with a horny covering. It bears nothing so far as I could discover that would aid in holding it fast. Several capsules were opened carefully by means of sharp needles and the contents examined. There were perhaps a score of eggs and many nutritive cells.

Summary.

1. *Planaria simplississima* produces winter eggs.

2. A single individual may develop three or more capsules during the winter.

3. Complete regeneration of tissue lacerated at the extrusion of the egg capsule is effected in about one week.

4. About three weeks elapse between two successive extrusions.

LYSOROPHUS, A PERMIAN URODELE.

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University of Chicago.

Thirty-one years ago Professor Cope described [1] briefly three small and incomplete vertebræ from the "Permian" of Vermilion County, Illinois, as those of a reptile under the name *Lysorophus tricarinatus*. The type specimen was figured, with additional descriptions, by Case in 1899.[2] In a later paper[3] Case recognized the same form from the Permian of Texas and gave a good description and figures of the vertebræ and ribs. From the peculiar coiled condition of the various intermingled series of vertebræ which he had himself collected he concluded that the animal was long and snake-like. No limb or pectoral or pelvic bones have ever been detected. Associated with the form but not definitely connected with the vertebræ was a fragment of the skull of a small animal which he doubtfully referred to the same species, but which he also was inclined to refer to *Isodectes* Cope. In 1904 Broili [4] with real skull material and less perfect vertebræ, reached the startling conclusion that the genus showed certain affinities to the fishes, because of the presence of what he thought were gular plates in the palatine region. Chiefly because of their supposed presence he proposed the family name Paterosauridæ for the genus, which he located in the Rhynchocephalia. It is needless to say that his views of the diphylectic origin of reptiles, one phylum directly from the fishes, the others from the amphibians, has been received by naturalists with doubt and incredulity, and are, as will be seen, wholly unsubstantiated by this animal. His "gular plates" were doubtless merely misplaced proatlas bones. It is rather surprising that he should have overlooked the almost impossible reptilian char-

[1] *Proc. Amer. Phil. Soc.*, 1877, p. 187.

[2] *Journal of Geology*, V., p. 714, pl. II., ff. 12*a*, 12*b*, 12*c*.

[3] *Ibid.*, May, 1892, p. 46, pl. IX., ff. 1, 2.

[4] *Paleontographica*, LI.

acters of the vertebræ and skull, characters certainly impossible for a rhynchocephalian.

Recently, in examining the material in the Chicago collections, the remarkable characters of the vertebræ, so anomalous for any reptile, and utterly unknown in this class from the Permian otherwise, aroused my interest and doubt. From the matrix containing several series of vertebræ a corner of a bone protruded which I recognized as of a mandible. Under the skilful manipulations of Mr. Paul Miller a wonderfully complete and undistorted skull was brought to light. In similar matrix, and associated with vertebræ of the same kind I recognized another mandible and several small, pitted dermal bones, probably belonging to another type of amphibian, though it is not impossible the scutes were those of *Lysorophus*.

That the present species belongs in the genus *Lysorophus* from the reputed Permian of Illinois seems tolerably well assured, though the type material of the genus is rather scanty, and not entirely sufficient to resolve doubt. That the species are identical is I believe quite improbable. With this understanding, however, it will do no harm to use Cope's name for both genus and species until such time as more and better material of the species has been obtained from the original or contemporaneous beds.

Lysorophus tricarinatus Cope.

Skull (Figs. 1–3). — The general shape of the skull is that of a four-sided pyramid, pointed anteriorly. The upper surface is nearly plane, very gently convex from side to side, and also longitudinally in front. The sutures are widely separated in the specimen, indicating a loosely joined skull, and the bones are quite smooth, without pittings or mucous canals. The nasals are relatively large bones, with nearly parallel sides, rounded anteriorly. The frontals are also four-sided, the longer sides nearly parallel; the bone is about twice as long as wide. On either side, beginning at the transversely extended fronto-parietal suture, there is a narrow bone which seems to be continuous as a single element to beyond the middle of the nasals, ending acuminately in front. This is doubtless the prefrontal of the modern urodeles. The parietals are broad and large bones, like

the frontals with nearly parallel sides, overhanging, for the most part, the open temporal region. There is no parietal foramen. They are gently convex from side to side. From this posterior suture the upper surface of the skull turns downward at an angle of about twenty degrees, so as to bring it nearly parallel with the plane of the lower margins of the mandibles. Three rather large bones are seen here, a median unpaired one and two larger lateral ones. The median bone is broader in front than behind and borders the large foramen magnum; it doubtless corresponds to the median cartilage found in many urodeles, called the supra-occipital usually, by Gaupp the tectum synoticum. The lateral

FIG. 1. *Lysorophus tricarinatus*, skull from above, enlarged three diameters.

bones are larger, of an irregularly square shape with a hook-like prolongation exteriorly behind, turned downward back of the squamosal. They must be identified as "epiotics," a bone rarely if ever found separate in the modern batrachians. The squamosal (paraquadrate of Gaupp) unites, by a nearly straight antero-posterior suture, with the parietal and epiotic and then turns downward, forward and a little outward, narrowed and more rod-like below. There is some doubt of its union with the quadrate, but the division seems apparent on each side. With this interpretation, the quadrates are small bones, about twice as

long as wide terminating in the cotylus, and perforated a little above the lower extremity by a foramen. The double occipital condyles are sessile, each with an oblique, flattened articular face looking inward and backward. Just in front of the condyles exteriorly there is a small foramen for the vagus, in front of which there is a large vacuity for the ear opening, partially or imperfectly closed in front by this combined bone. Above, the bone sends a triangular prolongation inward to the lower edge of the supraoccipital, bordering the hind margin of the epiotic. The large foramen magnum is thus bordered as in modern urodeles, nearly completely, by the exoccipital. Rather closely applied to this margin is a pair of triangular bones meeting roof-like in the middle above and terminating below in an angle a little above the condyles. They occupy the position of, and doubtless are the so-called proatlas bones, displaced to form the "gular plates" of Broili. The basioccipital bone I at first thought to be ossified, but further examination convinces me that the broken surface seen in the specimen between the condyles below is the broken off anterior end of the atlas. A like condition was found by Broili in his specimens, but he interpreted the structure as that of a broken off occipital condyle. Furthermore, a little in front of this fractured surface is seen the hind margin of a thin transverse plate, the parasphenoids.

In the palatal region are lying four pairs of branchial bones, with no indications whatever of so-called gular plates. The position and relations of these bones are well shown in the accompany ing photograph (Fig. 3). The outer pair lying close to the inner margins of the mandibles, have the posterior end thickened and recurved, hook-like, to abut against or approach the hind side of the quadrate. I would take them to be ceratobranchials save for the fact that a pair of nearly square bones very clearly articulate with the anterior ends, which must be ceratobranchials. To the inner side, and progressively more posterior, lying symmetrically, are three pairs of epibranchials the inner and hindmost represented in the specimen only by their anterior ends, the posterior portions broken off with the altas. The two outer pairs, at least, are thickened and truncate at each end, and are partly hollowed or cancellated, like all other bones of the skeleton. The first of

these pairs also seems to have a thickened and recurved posterior extremity. The mandibles are rather stout, extending a short distance back of the cotylus, expanded and flattened, somewhat spout-like in front. Each bears about twelve, conical, simple teeth on the anterior, somewhat concave margin, which is about two

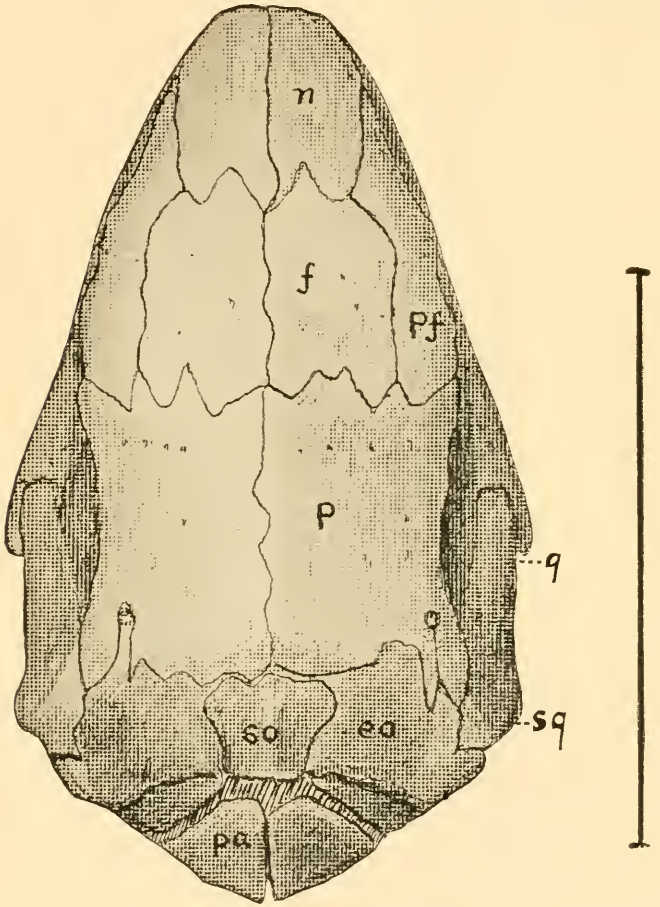

FIG. 2. *Lysorophus tricarinatus*, skull from above, enlarged five diameters. *n*, nasal; *f*, frontal; *pf*, prefrontal; *p*, parietal; *so*, supraoccipital; *eo*, epiotic; *pa*, proatlas.

fifths the length of the mandible; the teeth are directed somewhat obliquely outward.

The sides of the skull are in a nearly vertical plane, directed

somewhat obliquely outward posteriorly. The squamosal and quadrate, extending downward, forward and a little outward, meet the cotylus of the mandible a little back of the middle of the skull. Opposite the mandibular teeth in front are the narrow maxillæ, with teeth like those of the mandibles. They end freely and acuminately behind, and if connected at all with the bones of the upper part of the skull, the connection was small and slender and situated far forward. The whole side of the skull, from in front of the squamosal and quadrate seems to have been unossified; there are no jugals nor quadratojugals, and no temporal arches. The premaxillæ are also very slender, with four or five small teeth on each side. The position of the eyes was far forward, in the narrow space between the maxillæ and the prefrontals, and it is quite certain that these organs must have been very small. The nares also must have been minute and situated far forward, probably between the nasals and the premaxillæ near the middle line. Altogether, in life, save for its greater narrowness and more snake-like appearance the whole head must have been strikingly like that of *Necturus*.

Vertebræ. — The centrum is moderately elongated, deeply biconcave with persistent notochord, wholly without trace of hypocentra. In the middle below there is a median rounded keel, concave longitudinally, with a deep pit or fossa on each side reaching nearly to the internal cavity. On either side there is another, more slender carina, bounding the fossa above, with a more shallow concavity above it. The pedicel is elongate anteroposteriorly, the neural canal large. The centrum has no parapophysial facet or process for the rib. A little below and back of the anterior zygapophyses is the diapophysis, a flattened process directed anteriorly and a little downward, with the extremity thickened and a little rounded; they are short. The arches are depressed, a little convex in the middle anteroposteriorly, but without spine, the two sides separated by a persistent median suture, and the two bones are usually drawn somewhat apart, like the bones of the skull. The zygapophyses are rather large, flattened on their articular surface and are directed somewhat inwards or outwards. The ribs are large, flattened proximally, more cylindrical distally and are hollow. They have an

anterior angulation or curvature near the proximal third, this third being directed more obliquely backward. There are no traces of abdominal ribs in the numerous specimens examined, nor any of dermal plates, save in the case already spoken of, plates evidently belonging with a small mandible near them of an apparently different type from that of *Lysorophus*.

The terminal part of the tail, which is preserved in one speci-

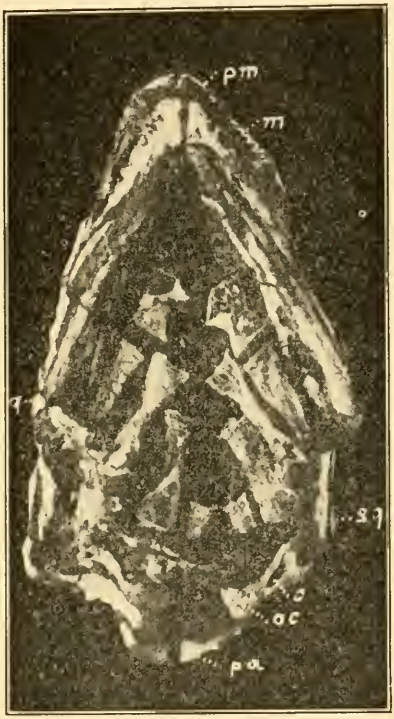

FIG. 3. *Lysorophus tricarinatus*, skull from below, enlarged five diameters. *pm*, premaxilla; *m*, maxilla; *sq*, squamosal; *o*, otic fenestra; *oc*, occipital condyle; *pa*, proatlas.

men, ends rather gradually, tapering to a point. In the seventeen vertebræ of the series there are no ribs and no diapophyses, or very rudimentary ones anteriorly; and I can find no traces of chevrons. Characteristic figures of the vertebræ and ribs will be found in the cited papers of Case.

That *Lysorophus* is not a reptile requires no argument — the

unpaired supraoccipital, the absence of pineal foramen, quadratojugals, jugals, postfrontals, temporal arches, the evidently large parasphenoid, the double occipital condyles, paired branchials, neurocentral, single-headed ribs, etc., are positive evidence that the animal is not only not a reptile but that it is related to the modern urodele amphibians. In skull structure the characters are urodelan in every detail save the separated "epiotics," the intercalary of Cuvier, Vrolik and Cope, the paroccipital plates of Baur, the posttemporals of Broom; and this separation is precisely what would be expected in the early urodele. The exoccipitals otherwise seem to be a single bone in *Lysorophus*, but it is very probable that they are the result of an early fusion of the exoccipitals, paroccipitals and proötics. The squamosals and quadrates have the position and relations of modern salamanders, the quadrate rather better ossified than is usually the case. That the supraoccipital should be ossified is, also, what might be expected. The remarkable fact that this bone should be unpaired while all the remainder of the bones of the skull are very loosely joined, as also the fact that the corresponding element in the urodeles is cartilaginous would seem to preclude its identification with the paired supraoccipital plates, the postparietals of Broom, of the Stegocephalia, rather favoring Gaup's contention of the nature of his *tectum synoticum*.

The tricarinate structure of the vertebral centra is quite aberrant for a reptile, but not remarkable for a urodele; so also is the sutural division of the neurocentra, and, for the Permian, the neurocentral attachment of the single-headed ribs. The only aberrant character to distinguish *Lysorophus* from the Urodela is the long and rather broad ribs, unknown among these modern animals or their possible ancestors the Branchiosauria. It is, however, very evident that the earliest ancestors of both these groups must have long ribs, and their persistence in *Lysorophus* would be nothing remarkable. Nor do I think it impossible that *Lysorophus* and its immediate kin may have developed long ribs from the earlier short ones. Certain it is that this character alone, and it is the only aberrant one, should not exclude *Lysorophus* from the Urodela, though it may necessitate a slight revision in the definition of the group. That *Lysorophus* cannot

be classed with any of the divisions of the so-called Stegocephalia is quite as evident as its exclusion from the Reptilia; and upon the ribs alone the formation of a new order of Amphibia would not be at all justified.

That the genus represents a distinct family of the Urodela is of course obvious. Broili, under the erroneous supposition that it is a reptile of the order Rhynchocephalia gave to it the name Paterosauridæ, to indicate its "paternal" relationship with the reptiles. But this name was chosen in direct contravention of the rules of zoölogical nomenclature, since it is not represented by a genus in the group, and since any one is at entire liberty to apply the stem as a generic name in another group. The name is not tenable, and should be replaced by Lysorophidæ.

The condition in which the remains of so many of these animals are found, numerous series intermingled in vertical and lateral curves, is I think conclusive evidence that death overtook the creatures in the drying up of ponds and pools of water. That the animals were snake-like in life is of course proven by the long connected series of vertebræ of nearly uniform size. That they had but feeble power of sight is also assured by the very small size of the eyes. That they were perennibranchiate is I believe also extremely probable from the large size of the branchiæ, and the manner in which these creatures represented by their known remains met their death. Doubtless also they were bare skinned and more or less mud burrowing in habit. That *Lysophorus* stood in direct ancestral relationship to such forms as *Necturus* or *Proteus* is rather improbable, but that it was very close of kin to the ancestors of these forms I do believe to be very probable.

Salamander-Like Footprints from the Texas Red Beds. (Fig. 4.)

Recently, Miss Augusta Hasslock, of the Abilene, Texas, High School, has had the kindness to send me a number of thin red shales showing abundant markings of raindrops, worm or other tracks and footprints which must have been made by some salamander-like creatures of small size. The horizon is assumed to be Permian, but the fact that the shales occur not far below the

Cretaceous, inclines me to the belief that it will eventually be found to be Triassic. Enlarged photographs are given of some of the best of these numerous prints. The reverse of those at *a* and *b* are shown in a^1 and b^1. In the lower, left corner is shown the figure of a much larger print. It will be observed that the prints occur in pairs, one with clear evidence of five, the other with but four toes, from which the conclusion is justified that the crea-

FIG. 4. Footprints from the red beds of Texas, near Abilene, enlarged about one third.

tures were tetradactyl in front, pentadactyl behind, as were the Branchiosauria and as are the Urodela of to-day. Whether or not they were real salamanders which made the prints, or branchiosaurs, cannot be determined, but I am inclined to believe, in the light of the evidence presented by *Lysorophus*, that the origin of

some or all of these prints is due to real salamanders of modern type.

VENTRAL RIBS IN LABIDOSAURUS INCISIVUS. (Fig. 5.)

In a recent paper[1] I stated my belief that ventral ribs would eventually be found in some of the forms now classed in the rather heterogenous group known as the Cotylosauria, and I suspected that the specimen of *Labidosaurus* therein described presented such evidence, but could not be sure. This evidence has, however, been made clear by further preparation of that specimen. In the removal of the matrix from the under side of the pubes a small fenestra of accidental origin was found, and in this

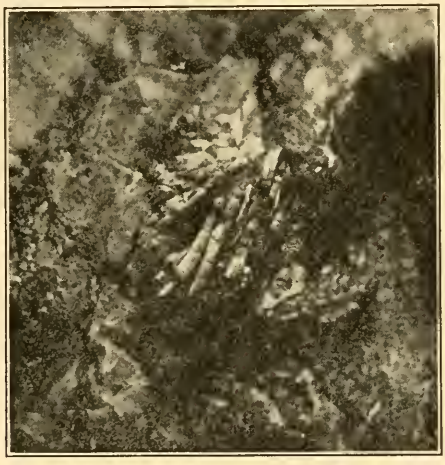

FIG. 5. Ventral ribs of *Labidosaurus incisivus*, enlarged about two diameters.

space, that is originally between the front end of the plate-like pubes and the vertebræ, are seen abundant evidences of small slender ribs, of some of which I give herewith a photographic illustration. At this spot seven ribs are seen lying closely together and parallel, directed from the anterior outer corner of the pubis inward and backward. Still further forward, and the continuation of these series, are further evidences of the same sort. The ribs are much smaller than I had expected to find them, but it is clear to me that the whole under side of the abdomen was enclosed in a closely set armor of slender ossified ribs. This char-

[1] *Journal of Geology*, XVI., p. 148, 1908.

acter adds another evidence of the relationship between the Procolophonia and *Labidosaurus*, and destroys its value as a group distinction.

Addenda. — Texas Permian Fields, September 25. Since the manuscript of the foregoing left my hands I have seen the recent paper by Case (Bull. Amer. Mus. Nat. Hist., xxiv, 531, June 30, 1908), in which he recognizes the amphibian nature of *Lysorophus*, figuring the skull with its exposed palate. He does not, however, discuss the relationship of the form and we differ in the interpretation of some of the bones.

Within the past week Mr. Miller, my assistant, has discovered two deposits of the species herein described from which cartloads of the peculiar nodules might be had for the digging. In two other places I have found them less abundantly. In examining the selected material, I have detected a small limb, very *Necturus*-like with four metapodials and epipodials in place, the mesopodials evidently unossified. It is of course possible, but not probable, that the limb is an accidental intrusion of some other small amphibian. My conviction is that the Lysorophidæ should be included in the Ichthyoidea.

FURTHER STUDIES ON THE ELIMINATION OF THE GREEN BODIES FROM THE ENDODERM CELLS OF HYDRA VIRIDIS.

D. D. WHITNEY.

In a recent paper I called attention to the fact that when green hydras are kept in a 0.5 per cent. solution of glycerine for several days they gradually lose their green color and become colorless.[1] The green bodies were observed to be thrown out of the enteric cavity through the mouth but it was not determined how they became separated from the endoderm cells in which they are contained. It was a matter of conjecture whether the endoderm cells became detached from the walls of the enteric cavity and carried the enclosed green bodies with them, subsequently disintegrating and liberating the green bodies in the enteric cavity, or whether the endoderm cells became ruptured and let their contents flow into the enteric cavity and then out through the mouth.

A microscopical study of the endoderm cells of both the normal green hydras and the green hydras that had been in a 0.5 per cent. solution of glycerine from one hour to about three weeks gave the following results:

In the normal hydras the endoderm cells are about as long as broad and each contains a large vacuole at its inner end. Nearly all of the green bodies are at the base of the cells. Figs. 1 and 2 show respectively in a cross and a longitudinal section the condition of the endoderm cells in green hydras that were starved for fourteen days in clear water. Several which were starved only thirty-six hours were sectioned but the endoderm cells did not differ noticeably from those in the green hydras that were starved for two weeks. The animals were allowed to remain without food in order that the endoderm cells, free from food, might be compared with those of animals that had been in the glycerine solutions for the same length of time without food.

[1] Biological Bulletin, 1907, XIV.

When green hydras are put into the glycerine solution the endoderm cells become larger. As the cells are closely packed together their expansion laterally is prevented. Consequently becoming larger they push out into the enteric cavity, becoming several times as long as in the normal animals. Fig. 3 shows the condition of the endoderm cells of a green hydra that had been in the glycerine solution for one hour. Some of the cells are about twice the length of those in normal hydras but otherwise they seem to be similar.

In Fig. 4 the endoderm cells are much longer and more narrow than in the preceding case. This hydra had been in glycerine solution for three hours. In Fig. 5 the endoderm cells are about the same size as in Fig. 4 but the interior of the cells is filled with a very fine granular substance and the green bodies are scattered about in this substance especially in the distal two thirds of the cells. Very few green bodies were seen near the free end of the cells. This section was from a hydra that had been in the glycerine solution for sixty hours.

The condition of the endoderm cells of hydras that were rendered entirely colorless by being kept in the glycerine solution for eighteen days or more, Fig. 6, differs only from the endoderm cells of hydras that had been in the glycerine solution for sixty hours in having no green bodies in the cells.

Each endoderm cell doubles its size at least within an hour after being put into the glycerine solution as is seen in Fig. 3. As it remains longer in the solution it becomes extended until it is ruptured and owing to the pressure of the adjacent cells extrudes much of its contents into the enteric cavity. As soon as there is an equilibrium of pressure the rupture quickly heals or regenerates thus making the cell intact again. Soon after it is whole it swells again until it is ruptured a second time and discharges more of its contents, including the green bodies, into the enteric cavity. This process is repeated as long as the animal is kept in the glycerine solution and if kept too long until its death. By this repeated process of the rupturing of each endoderm cell and the discharge of its contents all the green bodies of each cell are finally eliminated and the cells remain colorless.

The green bodies have been seen being ejected through the

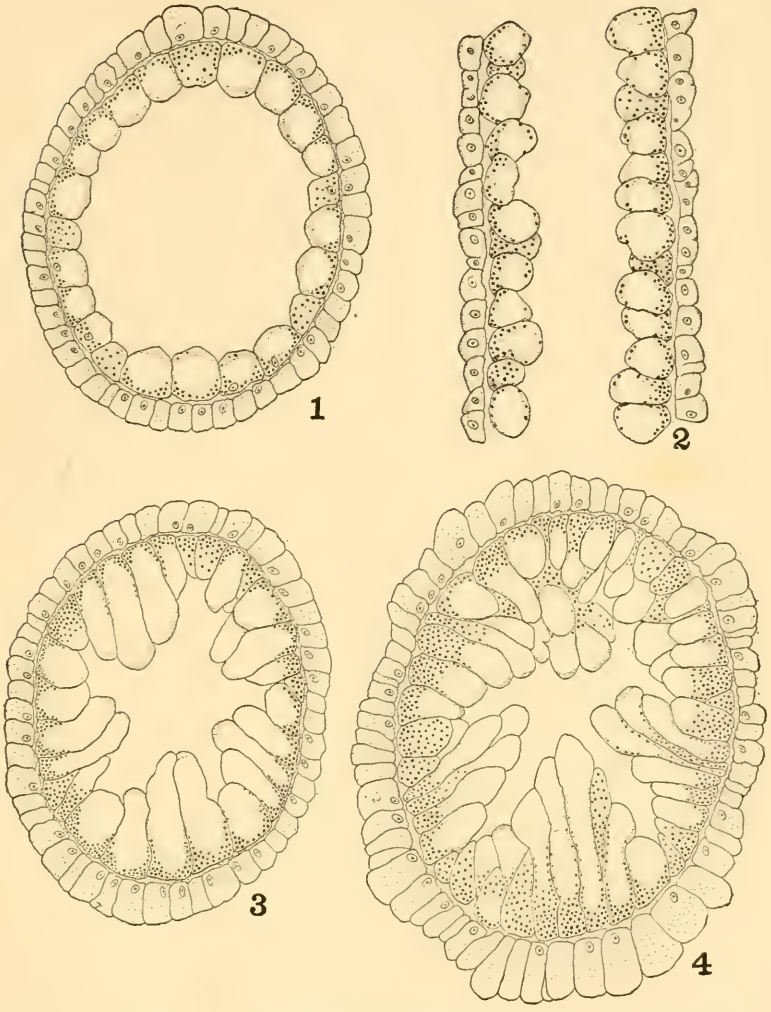

FIG. 1. Cross section of a green hydra that had been without food for 13 days.

FIG. 2. Longitudinal section of a green hydra that had been without food for 13 days.

FIG. 3. Cross section of a green hydra that had been without food for 36 hours and then put into a 0.5 per cent. glycerine solution for 1 hour.

FIG. 4. Cross section of a green hydra that had been without food for 36 hours and then put into a 0.5 per cent. glycerine solution for 3 hours.

mouth of living hydras which were in glycerine solution. They were also found in greater or less numbers in the enteric cavity

of the hydras that were sectioned and studied. They always seem to be free in the enteric cavity but sometimes they are mixed with a substance which resembles the granular contents of the endoderm cells. No free endoderm cells were ever seen in the enteric cavity.

Professor W. J. Gies was kind enough to perform some experiments for me in which blood corpuscles were put into a 0.5 per cent. solution of glycerine made with physiological salt solution. Upon measuring the diameters of the corpuscles both before and after putting them into the glycerine solution it was determined that if there was any change it was a slight shrinkage but never any enlargement of the corpuscles.

When these results of the effect of glycerine on blood corpuscles are compared with those obtained on the endoderm cells of hydras which were also kept in the same percentage of glycerine solution it is seen that the results are opposite — the corpuscles shrink and the endoderm cells become larger.

The shrinking of the corpuscles is probably due to an increase of the osmotic pressure of the solution caused by the addition of the glycerine and is purely a physical change.

The enlargement of the endoderm cells of hydras might be explained as due to the glycerine acting as a stimulus to the cell and causing certain vital processes in it to become active which result in a large and rapid absorption of water by each cell. The cells react to this stimulus as long as the animals are kept in the glycerine solution. Thus this change could be called a physiological one brought about by the stimulation of living processes in the cells.

Some cells were seen which had little protuberances or outpocketings on their inner free ends which looked as though they might be weak places in the cell wall that were forced out by the increasing pressure from within. Doubtless the ruptures occur at these places.

As it is a well-known fact that hydras have extraordinary powers of regeneration in the closing of wounds, as when their bodies are cut into two or more parts, and also in the replacing of lost parts, the assumption that the ruptured places in the endoderm cells close and grow together quickly is not, I think, an improbable assumption.

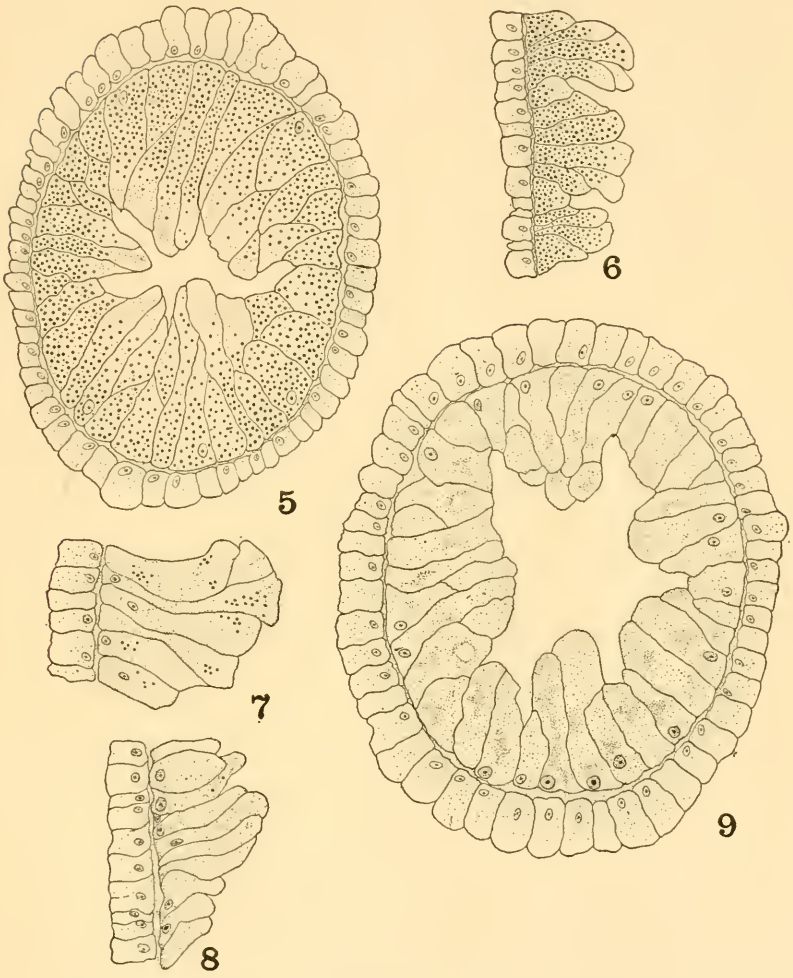

FIG. 5. Cross section of a green hydra that had been in a 0.5 per cent. glycerine solution for 60 hours.

FIG. 6. Portion of a longitudinal section of a green hydra that had been in the glycerine solution for 60 hours.

FIG. 7. Portion of a longitudinal section of a hydra that remained slightly green after being in a 0.5 per cent. glycerine solution for 13 days. Several groups of green bodies are seen in the endoderm cells.

FIG. 8. Portion of a longitudinal section of a hydra that was green when put into a 0.5 per cent. glycerine solution in which it remained 13 days and was rendered colorless. Only two green bodies seen in this section.

FIG. 9. Cross section of a hydra that was green when put into a 0.5 per cent. glycerine solution in which it remained 18 days and was rendered colorless. No green bodies are seen in this section.

Whatever the exact process may be it is certain that the endoderm cells remain in a greatly distended condition and lose all of their green bodies, many of which are found free in the enteric cavity.

Zoölogical Laboratory,
Columbia University,
New York City, May 29, 1908.

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BIOLOGICAL BULLETIN

THE HOMING OF THE BURROWING-BEES (ANTHOPHORIDÆ).

C. H. TURNER.

Introduction.

The researches about to be described were conducted for the purpose of determining how the burrowing bees compare with the ants and the mud-dauber wasps in their method of finding the way home. During most of the month of August, 1908, from five to ten hours a day were devoted to this study. This made it possible to conduct several series of experiments. Since all of the series led to similar conclusions, only two of them will be recorded. The majority of the experiments were conducted upon a species of *Melissodes* Latrl., many nests of which existed in an abandoned garden of the Haines Normal School.

Series A. Experiments on Melissodes.

These experiments were conducted in a deserted garden. Before beginning the experiments proper, numerous preliminary observations were made for the purpose of obtaining information that would be helpful in conducting and interpreting the experiments.

Bearing in mind Bohn's assertion that the flights of certain Lepidoptera are anemotropisms and phototropisms,[1] much attention was given to the flight of these bees.

When these anthophorids are busy at work, the flight is certainly neither an anemotropism nor a phototropism, for neither the movements nor the orientation of the body bear any constant relation to either the direction of the wind or to the rays of the sun.

[1] M. Bohn, "Observations sur les Papillons du Rivage de la Mer," *Bull. de L'Institut Général Psychologique*, 1907, pp. 285–300.

Observation soon informed me that each burrow was visited by bees at approximately regular intervals. Some of the nest-holes were visited by a bee about once in twenty minutes, other nests were visited more frequently. I soon discovered that where each interval between the visits was much less than twenty minutes, two or more bees occupied the burrow in common;[1] but that where the interval was twenty minutes or more, then only one bee was occupying the burrow. This enabled me to select, with a certainty, burrows that were occupied by only one bee.

The following series of experiments was performed upon a bee that occupied a burrow all to itself. The burrow was situated in a small barren spot and surrounded by a few blades of grass, which partially covered the opening. The heads of several stalks of grass overlapped the barren spot. The bee arrived at 9:35 A. M. and immediately entered the burrow. At 9:37 A. M., it departed again for the field, without stopping to explore the surroundings of the nest-opening.

EXPERIMENT 1.

While the bee was afield, a rectangular piece of white paper, 12 cm. by 8 cm., in the center of which was a hole 13 mm. in diameter, was so adjusted over the nest as to have the hole in the paper coincide with the opening of the burrow.

At 9:55 A. M., the bee arrived with its burden of pollen. Instead of entering the nest, it circled around and around. It then hovered, momentarily, over the white rectangle and then described yet wider circles in the air. This behavior was repeated several times. At 9:57 A. M., two minutes after its return from the field, the bee entered the nest. On again departing for the field, at 10:00 A. M., the bee hovered a while above the paper that surrounded the nest; then, after making several turns of a helicoid curve, flew away.

[1] To determine how many bees were occupying a burrow, I would plug the opening and then observe it carefully for an hour or longer. The bees, on returning, would circle about the nest. After a while they would usually try to dig around the plug. By counting the bees that appeared and tarried it was easy to determine how many bees were occupying the burrow. When the required information had been obtained, the plug was removed.

Experiment 2.

The same conditions as in experiment one.

At 10:20 A. M., the bee arrived from its trip, hovered for less than half a minute and then dropped into the nest. At 10:24 A. M., the bee departed, without stopping to explore the surroundings of the nest.

Experiment 3.

About four inches to the east of the nest opening, a hole was made in the ground. Over this hole was placed the piece of white paper, with the hole in the center, which was adjusted over the nest in experiment two. A piece of water-melon rind, with a thirteen mm. hole in the center, was so adjusted over the nest as to have the hole in the rind coincide with the opening of the burrow. One half of the rind was brown, the other half yellowish green; the line dividing these two colors bisected the hole in the center of the rind.

At 10:47 A. M., the bee arrived with its burden of pollen. It hovered above the melon-rind for a moment, then circled about the place. At 10:48 A. M., after a search of one minute, the bee entered the nest. On leaving the nest at 10:59 A. M., the bee examined carefully the surroundings before departing.

Experiment 4.

While the bee was afield, the piece of water-melon rind was removed and a rectangular piece of white paper, eight cm. long and five cm. wide, was arched over the nest in such a way as to form a tent six cm. high, the east and west ends of which were open. The rectangular piece of white paper, with the hole in the center, which was left in the same position as in experiment three, was situated just in front of the eastern opening of the tent.

When the bee arrived, at 11:15 A. M., it circled about for two minutes [until 11:17 A. M.] and then dropped into the hole over which the rectangular piece of paper, with the hole in its center, had been adjusted. It emerged at once and, after circling about for a short time, reëntered the same hole. It emerged immediately. Finally, at 11:18 A. M., three minutes after arriving on the spot, the bee entered the tent, through the eastern opening, and dropped into the burrow. On emerging from the nest, at 11:31 A. M., the bee hovered a moment inside of the

tent. It then passed out of the east opening and hovered for a few seconds above the tent. Then, keeping close to the top of the grass, it flew about for a while in a sub-helicoidal curve and then flew away to the field.

EXPERIMENT 5.

The same conditions as in experiment four.

At high noon, the bee arrived at the southern end of the tent. After hovering but a moment, it flew around to the front and entered the tent through the eastern opening. It then right-about faced and dropped into the burrow. On leaving, at 12:08 P. M., it hovered a moment inside of the tent, then departed without further exploration.

EXPERIMENT 6.

The same conditions as in experiments four and five.

On arriving from the field, at 12:28 P. M., the bee flew immediately, over the top of the tent, to the eastern opening and then directly to the burrow. It did not turn about before entering the nest. At 12:34 P. M., it departed without exploring the surroundings; it did not even hover in the inside of the tent.

EXPERIMENT 7.

The same conditions as in experiments four, five and six.

At 12:52 P. M., the bee appeared at the eastern entrance to the tent. Immediately the bee entered the tent and alighted on the ground. At once it flew upward and dropped into the burrow. At 12:59 P. M., it departed, without exploring the surroundings.

EXPERIMENT 8.

The rectangular piece of white paper, with a hole in its center, was left in the same position as in experiments five to seven inclusive; but the tent, over the nest opening, was so adjusted as to have its open ends face north and south.

At 1:25 P. M., the bee arrived at the eastern end of the tent. It immediately flew around to the southern entrance of the tent and entered the burrow.

The above eight experiments were performed August 14, 1908. On the next day (August 15) the following five experiments were performed with the same individual.

Experiment 9.

The same conditions as in experiment eight. The apparatus had been left in position for twenty hours. Something had trampled the tent to the ground. It was readjusted to the proper height.

At 9:34 A. M., August 15, the bee arrived, hovered but a moment, and then entered the tent, through the southern opening, and dropped immediately into the burrow.

Experiment 10.

While the bee was afield, several stalks of grass were removed from the west side of the tent; thus increasing the width of the barren patch that surrounded the burrow. The other conditions were the same as in experiment nine.

At 9:54 A. M., the bee arrived from the field. It circled about fully a minute before entering the tent. It then passed through the northern opening and immediately dropped into the burrow. On departing, at 9:59 A. M., the bee did not stop to explore the surroundings.

Experiment 11.

The same conditions as in experiment ten.

On arriving, at 10:18 A. M., the bee circled about for a moment and then, entering the tent through the southern opening, immediately dropped into the burrow. At 10:23 A. M., the bee departed, through the southern opening, without stopping to explore the surroundings.

Experiment 12.

While the bee was afield, the tent, with the open ends facing north and south, was placed two inches to the west of the nest-opening. A rectangular piece of black paper, 12 cm. long by 8 cm. wide, with a hole 13 mm. in diameter in its center, was so adjusted over the nest as to have the hole in its center coincide with the opening of the burrow. The rectangular piece of white paper, with the hole in its center, was left in the same position as in experiment eleven.

At 11:16 A. M., the bee arrived from the field, hovered a few seconds above the black paper, then dropped into the nest. On leaving, at 10:51 A. M., it hovered a short while before departing.

Experiment 13.

While the bee was afield, the rectangular piece of white paper was so adjusted over the nest as to have the hole in its center coincide with the opening of the burrow. The rectangular piece of black paper was placed in the position occupied by the white rectangle in experiment twelve. The tent was left in the same situation as in experiment twelve.

On arriving, at 11:16 A. M., the bee hovered above the white paper, over half a minute, before dropping into the burrow. On departing, at 11:23 A. M., it hovered quite a while, examining the surroundings.

Experiment 14.

While the bee was afield, all accessories were removed from the neighborhood of the nest and the barren patch covered with a thin layer of freshly mown grass. Care was taken to leave the opening to the burrow uncovered.

The bee arrived from the field at 11:50 A. M. and began to fly about in a sub-helicoidal curve. The radii of this curve became, irregularly, longer and longer until the bee had reached a fence fifteen feet away. Then the bee approached the nest and flew about in curves, the radii of which became, irregularly, shorter and shorter. At 11:52 A. M., after a search of two minutes, the bee dropped into the nest.

Series B.

The bee upon which this series of experiments was conducted was a much smaller insect than the *Melissodes* sp.? upon which the above experiments were performed. One of those tragedies, which are so common in the insect world, brought this bee's labors to a close before I was ready to capture it; hence it was impossible to determine the genus to which it belongs. I am not even sure whether it is a member of the Anthophoridæ or of the Andrenidæ. In this connection, however, the exact name is a matter of little weight; for, although there are generic, specific and individual peculiarities of behavior, yet the general habits of all the burrowing bees are so similar, that it would be illogical to suppose that the method of finding the way home was not essentially the same in all genera.

Several of the walks of Haines Normal School, Augusta, Ga., are separated from the adjacent flower beds by bricks inclined in such a manner as to form a serrated border of wedges of bricks; each wedge being about two inches high and something over four inches wide at the base. One of these flower beds, which was quite sandy, contained, in its center, a patch of nasturtiums. About two feet from the bricks, and parallel to the border, there extended, throughout the bed, a narrow row of violets. The remainder of the bed was bare. In a barren spot in this bed, adjacent to an inverted tin cap of a coca-cola bottle, and within an inch of the northern face of one of the bricks that formed the serrated border, a burrowing-bee had excavated a burrow. The nest was discovered at nine A. M., August 8, 1908. The sun was shining brightly at the time; but the nest, which was situated a little to the west of the southern wall of a large three-story brick building, was in the shadow. A gentle breeze was blowing from the south. At the time mentioned, the bee was busy collecting pollen and storing it in the burrow. The flowers from which it obtained its supply must have been quite remote, for it required about thirty minutes to make a trip.

For convenience, the brick before which the burrow was located was designated zero and bricks to the west of it W_1, W_2, W_3, etc., in regular succession. Likewise the bricks to the east were named E_1, E_2, E_3, etc.

The field from which the bee obtained its pollen was situated to the south of the school, and the burrow of the bee was located to the north of the brick border. On arriving from its forage, the bee would reach the brick border at, or near, brick W_{10}. It then would turn about so as to face the northern surface of the brick border. Then hovering at about an inch and a half from the ground and about the same distance from the bricks, the bee would sidle along. Usually its movement was toward the east; but, occasionally, it would retrograde westward a short distance and then resume its eastward progress. On reaching the brick before which its nest was located, it would drop immediately into its burrow. After remaining in the burrow a few minutes, the bee would depart, without stopping to explore the surroundings. Several trips of the bee were observed carefully and in

each case the behavior was essentially the same. In its flight, neither the orientation of its body nor the direction of its movements bore any constant relation either to the direction of the wind or to the rays of the sun.

Experiment I.

While the bee was afield, with a stick of the same diameter as the burrow, I punched, in the ground in front of bricks W_1 and E_1, holes which bore the same relation to each of those bricks that the burrow opening did to brick zero. The inverted tin cap of a coca-cola bottle was removed from its place beside the burrow and placed, in the same relative position, at the side of the hole which I had made in front of brick W_1.

The bee, on returning from the field, arrived at brick W_{10}. It then turned around so as to face the northern surface of the brick border. Then hovering at about an inch and a half above the ground and at about the same distance from the border, it sidled along. Most of the time it moved towards the east; but, occasionally, it retrograded westward, for a short distance, and then resumed its eastward progress. On reaching brick W_1, it dropped, at once, into the hole which I had made. It emerged at once and continued its eastward course until it reached its burrow, which it entered. It tarried in the nest a few minutes, then departed, without stopping to explore the surroundings, for the pollen field. Evidently, a slight topographical change of the neighborhood of the nest caused the bee to enter a false burrow, which it discovered was not its own.

Experiment II.

While the bee was afield, I punched holes, similar to those described above, before bricks W_2, W_3, W_4, W_5, and bricks E_2, E_3, E_4 — one hole before each brick. For descriptive purposes, I shall call the holes before bricks W_1, W_2, W_3, etc., respectively L_1, L_2, L_3, etc., and those in front of bricks E_1, E_2, E_3, etc., R_1, R_2, R_3, etc.

On returning from the field, the bee arrived at brick W_{10}. It then turned about so as to face the northern surface of the border, and, hovering and sidling, in the manner described in experiment I., it moved eastward until it reached hole R_1, into which it

dropped. Emerging at once, it hovered a moment and then dropped into the same hole. Again emerging it moved westward and dropped into its burrow. On emerging from the nest, it went immediately afield.

Experiment III.

While the bee was afield, I placed, before each of the holes I had made, except holes R_3 and R_4, an inverted tin cap of a coca-cola bottle. The other conditions were the same as in experiment II.

On returning from the field, the bee arrived at brick W_9. It turned about so as to face the northern surface of the border, and, in the hovering and sidling manner mentioned above, moved eastward, hovering momentarily over holes L_4, L_3, L_2, L_1, until it reached the nest, which it entered immediately. There it tarried a moment, then departed, without stopping to examine the surroundings, for the pollen-fields.

Experiment IV.

While the bee was afield, I placed a small tent of white paper over the burrow. The tent, the whole north end of which was open, was three inches wide, at the base, two inches high and three inches long. The other conditions were the same as in experiment III.

On returning from the field, the bee arrived at brick W_8. It then turned about so as to face the northern surface of the border and then, in the hovering and sidling manner mentioned above, it moved along, hovering, in the order mentioned, above holes L_4, L_3, L_2, L_1. On reaching the tent, it retraced its steps, hovering over holes L_1, L_2, L_3, L_4. It then resumed its eastward journey. Although it had been sometime since the bee returned from the field, yet it had not entered any hole. At this stage, however, it dropped into hole R_1. Emerging, it hovered a moment and then reëntered the same hole. Emerging from the hole, it began to fly about in a random manner. Evidently it could not locate the burrow. It had passed over the tent several times, but had made no attempt to enter it.

I now removed the tent, thus leaving everything in the same condition as in experiment III.

In a few moments the bee reached the nest and, after hovering

a moment, entered. On departing for the field, it spent considerable time hovering about the burrow, as though it were examining the surroundings.

EXPERIMENT V.

While the bee was afield, a rectangular piece of paper, 12 cm. long by 8 cm. wide, in the center of which was a hole 13 mm. in diameter, was so adjusted over the nest as to have the hole in the paper coincide with the burrow-opening. The other conditions were the same as in experiment three.

On returning from the field, the bee arrived at brick W_{12} and turned about so as to face the northern surface of the border. In the hovering, halting, manner mentioned above, it sidled eastward, hovering a moment over each hole reached, but entering none. Over the nest it hovered a little longer than it did over holes L_4, L_3, L_2, L_1; but, instead of entering, it continued its search eastward. On reaching hole R_1 the bee dropped into it. Immediately it emerged, hovered a moment, then dropped again into the same hole. Reëmerging from hole R_1, it journeyed eastward and dropped into hole R_2. Emerging from this hole, it passed to brick zero, hovered for about a minute above the burrow but did not enter. It now began to roam about at random. After the lapse of some time, it reappeared above the nest, hovered a moment and then dropped into the burrow. On emerging from the nest, the bee hovered about for some time and then circled about the neighborhood, before departing for the field.

EXPERIMENT VI.

The same conditions as in experiment V.

On returning from the field, the bee arrived at brick W_{10}. In the hovering, halting, manner described above, it sidled eastward, halting over each hole, but entering none. It started to enter hole L_1, but retreated before the body was three fourths hidden. As soon as the nest was reached, the burrow was entered.

CONCLUSIONS.

It is evident that the behavior exhibited by the above experiments cannot be classed as either anemotropisms or as phototropisms, for neither the orientation of the body nor the direction

of flight bore any constant relation either to the direction of the wind or to the rays of the sun. Many of the nests observed by me were in the sunshine a part of the day and in shadow the balance of the day, yet the bee found the nest just as readily when it was in the shadow as it did when it was in bright sunlight.

Any pronounced change made in the topography of the vicinity of the nest, while the bee is away from its burrow, is sure to cause the insect, on its return, to be forced to search about in order to find the entrance to its home [Ex. 1, 3, 4, 10, 14, I., II., IV., V.]. This is true even when the nest opening is in full view [Ex. 1, 3, I., II.]. If the proper alterations are made in the topography of the vicinity of the nest, the bee may be induced to enter, temporarily, a false burrow [Ex. 4, I., II., IV., V.]. A bee that has not been experimented upon is much more affected by slight alterations made in the topography of the vicinity of the burrow than is the same bee after a prolonged period of experimentation [cf. Ex. 1 with Ex. 13 and 14]. All of these statements militate against the old idea of a "homing instinct," against Pieron's kinesthetic reflex hypothesis and against Bethe's contention that bees are guided home by an unknown force which acts reflexly; for if either of these assumptions were true, changes made in the topography of the vicinity of the nest should not alter the behavior of the bees.

It would be erroneous to claim that these burrowing-bees find their way home by the method of "trial and error," for there is no gradual "stamping in" of an appropriate response. When the bee, on returning home, finds the environment markedly changed, it searches until the opening of the burrow is found. Before departing again for the field, the bee makes a careful examination of the vicinity of the nest [Ex. 1, 3, 4, 13, IV., V.]. On its next return to the burrow, unless the environment has been changed in the meanwhile, the bee flies directly to the burrow in the minimal amount of time; there is none of that blundering into a solution which the method of "trial and error" demands [Ex. 2, 5, VI.].

By a process of elimination, the most consistent explanation of the above behavior is the assumption that burrowing-bees utilize memory in finding the way home, and that they examine

carefully the neighborhood of the nest, for the purpose of forming memory pictures of the topographical environment of the burrow. This assumption that the exploration of the vicinity of the nest is for the purpose of forming memory pictures is supported by the fact that such explorations are always made before beginning trips that immediately follow some pronounced change in the topography of the environment [Ex. 1, 3, 4, 13, IV., V.], and not when such changes have not been made [Ex. 2, 5, 6, 7, 10, 11]. Slight changes in the topographical environment of the burrow may, at times, effectively disturb the bee on its homeward journey, and yet not be sufficiently pronounced to cause the departing bee to pause and reëxplore the surroundings of the nest [Ex. I., II., III.].

Haines Normal School,
Augusta, Ga., September, 1, 1908.

OBSERVATIONS ON THE BEHAVIOR OF THE HOLOTHURIAN, THYONE BRIAREUS (LESEUR).[1]

A. S. PEARSE.

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I. Introduction.

The different classes of echinoderms show in a remarkable way the extreme variations which one fundamental plan of structure may undergo in order to become adapted to different conditions of existence. Developing from a bilaterally symmetrical pelagic larva, the adult echinoderm becomes an almost perfect type of radiate symmetry, except in those forms which have a bilaterality superposed secondarily upon the typical radiate plan of structure, so that the adult becomes bilaterally symmetrical only after it has passed through the more primitive

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 119.

bilateral and radiate stages. Correlated with the variations in structure which are found in this group of animals, are corresponding differences in locomotion, respiration, feeding and other life processes, and the behavior of echinoderms is therefore a matter of particular interest on account of the opportunity it offers to compare the reactions of nearly related forms which have somewhat different types of symmetry. The reactions of certain stelleroids and echinoids (which are typically radiate in structure) have been carefully studied, but no observations of behavior have been made on holothurians (which are more or less bilateral) except for the brief papers of Clark ('99) and Grave (:02, :05). The writer had the opportunity, during July and August of the present year, of observing the common sea-cucumber, *Thyone briareus* (Leseur), and though lack of time prevented the observations from being very extensive and left many questions untested, it is believed that there are some points of interest in what follows. The object of the work was (1) to determine what the normal activities of this holothurian are and (2) to discover how its reactions are influenced by external stimulation. The experiments were carried on in the Marine Biological Laboratory, at Woods Hole, Mass., and my thanks are due to the director, Professor F. R. Lillie, for his kindness during the work.

II. Structural Characteristics.

In order that we may have clearly in mind the structural peculiarities of the form with which we are dealing and to gain an idea of the points in which it has departed from the typical radiate plan of symmetry, some time will be devoted to a brief review of the anatomy. *Thyone briareus* (Fig. 1) is a spindle-shaped animal which varies considerably in size, and this variation is not only dependent on the age but also on the condition of contraction or expansion. Fully extended individuals sometimes measure over twenty centimeters in length and a specimen of this size would be only six or seven centimeters long when contracted. At the posterior end there is an opening through which water is drawn into the cloaca and expelled again for purposes of respiration and excretion. The mouth is at the anterior end and is surrounded by

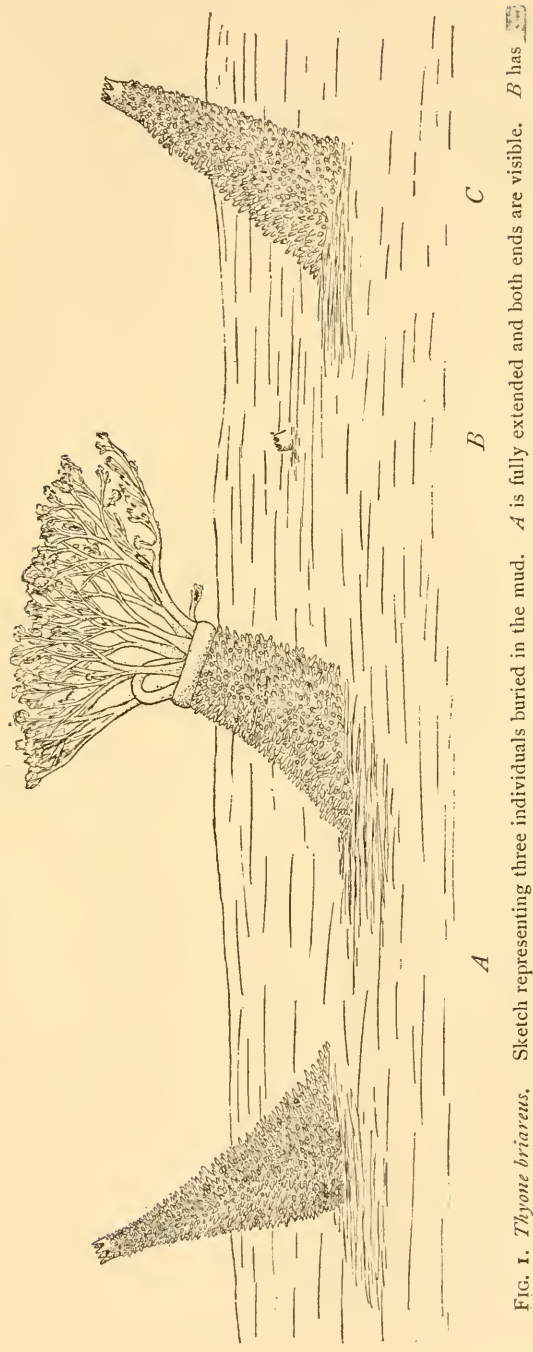

FIG. 1. *Thyone briareus.* Sketch representing three individuals buried in the mud. *A* is fully extended and both ends are visible. *B* has only the tip of the posterior end uncovered but *C* projects somewhat further above the mud.

ten dendroid tentacles which may be extended for feeding or collapsed and completely concealed by the turning in of the anterior end of the body. Just behind the tentacles is a "collar" which is without the slender tube-feet which cover the rest of the body. The tube-feet on the ventral and lateral surfaces have well developed sucking discs at their tips but such adhesive organs are not uniformly present on the dorsal side of the body. Besides this difference in the tube-feet, the dorsal and ventral surfaces may be distinguished from each other by the fact that the former is shorter than the latter and hence the ends of the body turn upward when the animal rests on its ventral side. They may be further distinguished by the difference in color (ventral being lighter), by the presence of a single median dorsal genital papilla between the bases of the tentacles, and by the fact that the two ventral tentacles are much shorter than the others. The internal organs are strikingly bilateral in their arrangement. This is shown by the right and left respiratory trees which unite and have a common opening on the dorsal side of the cloaca, by the single dorsal gonad and madreporite, and by the ventral Polian vesicles. The organs of the body wall are, on the other hand, typically radial in arrangement and hence the skeletal, muscular, water-vascular and nervous systems conform in general to the usual echinoderm type of structure. The water-vascular and nervous systems both consist of a circum-oral ring from which five radial branches extend in the body wall to the region of the cloaca. The skeletal parts consist of ten ossicles around the mouth which together form "the lantern," and of minute calcareous plates imbedded in the skin at the anterior and posterior ends of the body. The muscular system consists of five longitudinal bands, which extend from the circum-oral ossicles to the posterior end of the body, and of circular muscles which lie just beneath the skin and completely fill the space between the longitudinal muscles. The digestive tube is considerably coiled and is differentiated into four regions: an œsophagus which passes through the lantern, an expanded stomach, a slender intestine, and a musular cloacal chamber which gives rise dorsally to the respiratory trees.

From this brief synopsis of the structure of *Thyone*, it will be seen that, though we are dealing with an animal having a radiate

plan of structure, it has been modified in such a way that all of the systems of organs are more or less bilateral. There are well-differentiated anterior and posterior ends, but these are at the extremities of an axis which is horizontal instead of vertical as in most other echinoderms, for, like all holothurians, this species rests on its ventral side and the oro-anal axis is hence parallel to the surface on which it lies.

III. Methods of Response to Stimuli.

The behavior of any animal consists of the reactions which it gives in response to changes in its environment or to changes in its internal condition. It is an easy matter to change the surrounding conditions but we can interpret the internal changes which an animal undergoes as it responds to various stimuli only by its movements. Every species is limited to certain types of response by its structure, by the medium in which it lives, and by its past history. Therefore the first question to be considered is, by what reactions is *Thyone* able to respond when it is stimulated? The chief types of response will be stated briefly and the consideration of how they are brought about left for later discussion.

1. *Withdrawing Reaction.* — The reaction which is most often seen is the withdrawal of the posterior end of the body and the closing of the cloacal opening. The extent of this response depends upon various factors and it may be so slight as to be barely noticeable or it may be so marked that the body entirely disappears beneath the sand in which the animal is buried and remains out of sight for two or three minutes. Similar withdrawing reactions are performed by the anterior end of the body and also by the isolated parts, such as a single tube-foot.

2. *Extending Reaction.* — Under certain conditions the posterior end of the body becomes greatly elongated and is sometimes stretched as much as nine centimeters above the mud in which an individual lies buried.

3. *Locomotion.* — If *Thyone* is placed on a hard surface, such as the bottom of a glass dish, it attaches the tube-feet and moves across the surface and it may even climb the side of the dish. It is also able to burrow into sand or mud and may move about somewhat beneath the surface.

4. *Change in Size.* — As has been previously stated, this species undergoes marked changes in size and may shrink to a half or a third of its original volume when it is strongly stimulated.

5. *Feeding.* — Under certain conditions the circum-oral tentacles are extended and either waved in the water or swept over the surface of the mud in which the animal is buried. They are then consecutively poked into the mouth and wiped off. This reaction has been briefly described by Grave (:02).

6. *Change in Respiratory Movements.* — Water is periodically drawn through the cloacal chamber into the respiratory trees and expelled again. This series of breathing movements may be interrupted for a time or the rate may be increased or decreased.

7. *Self Mutilation.* — When the water becomes stagnant or when conditions become otherwise unfavorable the anterior end of the body is often cast off together with some of the visceral organs. The lantern, the circum-oral nerve and water vascular rings, the tentacles, and more or less of the enteric canal are frequently lost in this manner.

There are then at least seven well-defined reactions which may be used as a basis for the study of the behavior of *Thyone.* None of these responses are invariably called forth, however, when an individual is subjected to a certain stimulus. While one reaction is taking place it may exert an inhibitory influence on others, and the responses are all more or less changeable and therefore apt to vary in degree with a repetition of the same stimulus.

IV. Locomotion.

1. *On a Solid Surface.* — Individuals which were moving on a solid surface were never observed to extend the tentacles and remained more or less contracted so that they were usually not more than seven or eight centimeters long. When an animal is placed on the bottom of a dish in sea water it remains contracted for a short time, but the ventral tube-feet usually become attached within a minute. The posterior end is then slowly extended and the respiratory movements begin; the tube feet are protruded on all sides of the body and begin to wave about, and those which come in contact with a solid object attach themselves. The animal may move in any direction but the locomotion usually carries

it away from the source of the light, as *Thyone* is very sensitive to photic stimulation. Locomotion is brought about by shortening the tube-feet after they have been extended and attached, by twisting and extending movements of the whole body, and it is also assisted by sharp waves of muscular contraction which travel from one end of the body to the other.

The tube-feet act by pulling. They were never observed to become rigid enough to lift the body from the surface on which it rested, nor was there any pushing action, such as Jennings (:07, p. 99) described in the starfish. There was some lack of correlation in their movements and this manifested itself in two ways. When locomotion was taking place in a definite direction, the tube-feet were not only extended on the side towards which the animal was moving, but also over all the rest of the body. This was doubtless due partly to the fact that the tube-feet serve as organs of touch as well as of locomotion, but there were nevertheless a large number of seeking movements which were apparently of no use in locomotion. Furthermore, some of the tube-feet which were behind as an individual moved often remained attached for some time after they could be of any help in locomotion, and, after being greatly stretched, they were actually jerked from their attachments with a snap. They were never torn loose from the body, however, as often happens when *Arbacia* is pulled away from a solid surface. The stimulation which brings about the attachment of the terminal discs of the tube-feet is apparently contact with a solid object. Bits of shell, sand, and other bodies were frequently held by them for several days at a time.

In addition to the pulling action of the tube-feet, locomotion was often assisted by movements of the body. Individuals sometimes assumed a shortened form, detached the tube-feet at one end of the body, and then elongated this free end or made slow seeking movements which were somewhat like those of a leech. This free portion of the body was then attached and the animal slowly regained its contracted form again, thus making some progress. The sharply defined waves of contraction which commonly passed from one end of the body to the other were apparently of use chiefly in enabling the tube-feet to gain a new

attachment. A constriction might appear at either end as a ring around the body and pass to the opposite end. These rings usually moved over the body singly and a new one appeared about every three minutes on an active individual. Sometimes constrictions appeared simultaneously at both ends and neutralized each other as they met in the middle. As one of these constricted rings moved along the tube-feet were pulled from their attachments and folded into it, and when they were again extended they became fastened at points farther along in the direction of locomotion. As has been stated, *Thyone* apparently experienced some difficulty in getting the tube-feet to detach themselves at the proper time and this "ring-of-constriction" method was entirely effective in simultaneously pulling them loose in a certain region of the body. It is not intended to intimate however that the tube-feet could not be detached and moved forward without the use of these periodic constrictions.

Perhaps the most striking feature of the locomotion on a solid surface was the fact that it was without definite orientation. Individuals moved with the posterior end in advance as often as with the anterior end, and although the long axis of the body was as a rule approximately parallel with the direction of locomotion, animals often moved a long distance (as much as 12 cm.) with the body at right angles to the direction of movement, that is, they moved straight toward the right or left. The rate of locomotion was slow, the most rapid movement recorded being seven centimeters in fifteen minutes, or nearly half a centimeter per minute. In climbing up a vertical surface like the side of a dish the movements were not essentially different from those which took place when an individual was creeping on a horizontal surface.

2. *Burrowing.* — When *Thyone* is placed in a dish of sea water on a sandy bottom it usually twists and turns the body until it comes in contact with the side of the vessel. It attaches itself to the side, burrows downward, and then moves away from the side of the dish into the sand. The tube-feet are apparently of little use in locomotion on sand, and this fact supports the conclusion reached from watching their action on a solid surface, that they are effective in pulling the body along rather than in pushing

it. Occasionally individuals were found which burrowed directly into the sand without attaching themselves to any solid object. The results of an experiment performed on July 25 are typical of the other cases of burrowing observed. Four animals were placed in aquarium jars containing sand and sea water. One individual burrowed straight down into the sand and covered itself in three hours; another lay on top of the sand two hours and then took four hours to burrow; the third individual twisted about on top for half an hour, then came in contact with the side of the jar and burrowed into the sand in two hours and a half; the fourth took four hours to reach the side and then partly covered itself in two hours. On another occasion an animal lay on the sand eighteen hours but covered itself in two hours when placed against the side of the jar.

Burrowing is accomplished by a contraction of the body muscles and the action of the tube-feet. When the body is fastened to some solid object, the tube-feet are attached as far down its side as possible and the body is drawn out so that it is wedge-shaped in cross-section. The longitudinal body muscles are then contracted so that the body shortens, and the sand is forced aside as the cross-section becomes more circular in outline. During the entire process the two ends of the body are turned upward and the animal sinks down into the sand with the dorsal surface constantly uppermost. This method of procedure is usually repeated until the individual is completely buried, except the posterior end. The downward movement is assisted by the passage of constricted rings from one end of the body to the other, or from both ends toward the middle, the sand thus being loosened so that the body can be "wedged" down into it. When there is no solid object for the attachment of the tube-feet, burrowing is more difficult. As the waves of contraction pass over the body, the sand is gradually pushed aside so that a thin portion of the ventral surface is forced downward (Fig. 2, *A*). The dorsal longitudinal muscle bands and the circular muscles then contract and the ventral portion of the body expands in such a way that the sand is forced aside (*B*). This same process is repeated at intervals until the animal is covered.

Thyone can move about to some extent after it is buried in the

sand. It accomplishes this in the same way that it burrows and also to some extent by bending and straightening the body. A series of experiments was carried out to ascertain how deep *Thyone* could be buried in the sand and yet burrow out. The method was to place an individual in the bottom of a jar containing sea water to a depth of 55 cm., which was the height of the jar. It was then covered with sand to the desired depth. Individuals

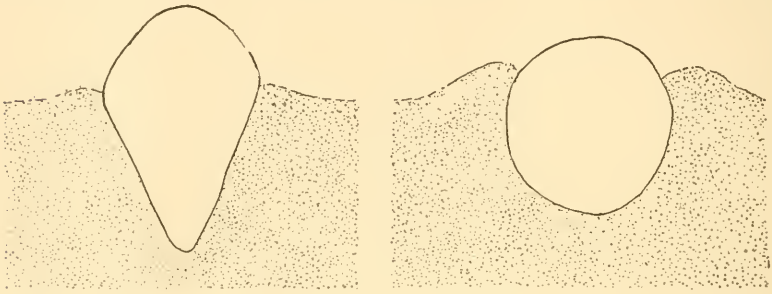

FIG. 2. Diagrams representing the shape of cross-sections in the middle of the body of *Thyone* as it burrows into the sand. The form shown in *A* precedes that shown in *B*.

were able to come to the top of the sand when covered under fifteen centimeters but none came up through twenty centimeters although ten large individuals were tested.

Clark ('99) found that *Synapta* burrowed with the tentacles and that it always went into the sand "head first." *Thyone* differs from it quite strikingly in the latter respect but this difference is perhaps no more than would be expected from the structural unlikeness between the two forms.

V. FEEDING.

After an individual has been undisturbed for some time it often extends the anterior end of the body and the tentacles and makes feeding movements (Fig. 1, *A*). In this extension the longitudinal muscles pull the lantern forward and the circular body muscles contract. The anterior end of the body is thus everted, like the turning inside out of the finger of a glove. The branched tentacles are then pushed out by the pressure of the fluids within them and the action of the muscles in their walls.

The two short ventral tentacles are most active and constantly move in and out of the mouth opening, while the larger tentacles wave about more slowly. The latter are moved through the water or scraped over the bottom and then consecutively wiped off in the mouth. When one of these large tentacles is wiped off, its proximal end is pushed into the mouth first and the distal branches follow. Before one tentacle has emerged, another is usually being pressed down upon it ready to enter. Sometimes two of the large tentacles bend toward the mouth at once but in no case were two seen to enter the mouth simultaneously, one of them always bending back after a moment to make way for the other. The eight large tentacles are used in a more or less regular sequence, and in general it may be said that the one which has been out of the mouth longest and which is farthest from the tentacle which is emerging will be the next to enter the mouth. They are seldom used in the exact order one would expect from this statement however. For example, those nearest the muddy bottom are usually more frequently used than the others. Many observations were made as to the sequence in the wiping of

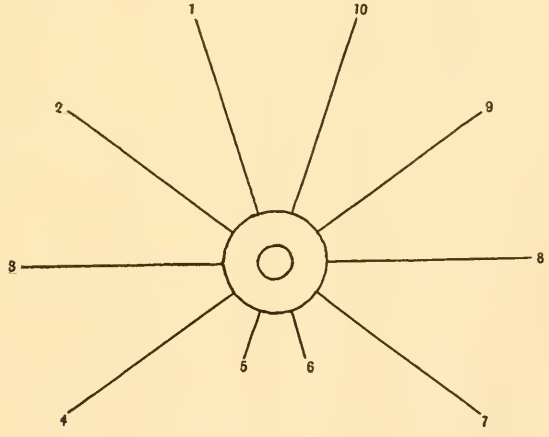

FIG. 3. Diagram to show the arrangement of the tentacles around the mouth as seen in an anterior view.

the tentacles and the following series is a typical one (see Fig. 3): 2, 7, 1, 4, 10, 8, 3, 9; 7, 2, 1, 4, 10, 8, 3, 9; 2, 7, 1, 4, 10, 8, 3, 9; 7, 2, 4, 1, 7, 10, 3, 4, 8; 2, 7, 9, 1, 3, 9, 7, 1, 8, 3, 10, 2, 8, 1, 7, 4, 8, 3, 10, 2. In this series a rather regular se-

quence is shown in the use of the tentacles. Number 7 was used eight times; numbers 1, 2, 3, 8, seven times; numbers 4 and 10, six; and number 9, five times. The time required for the fifty-three reactions was nine minutes and thirty seconds. A little over ten seconds was therefore required for the wiping of each tentacle. No account was taken of the two ventral tentacles in this series as they kept moving in and out almost constantly and without any apparent relation to movements of the others. In another larger series the different tentacles were used the following number of times: No. 1, twenty-one; No. 2, sixteen; No. 3, fourteen; No. 4, twelve; No. 7, eleven; No. 8, fourteen; No. 9, twenty-one; No. 10, nineteen. In this case tentacles 1, 10 and 9 were nearest the surface of the sand and they were used most frequently while those on the opposite side of the mouth (3, 4, 7) were less often employed. The time for this series of one hundred and twenty-eight reactions was fifteen minutes and thirty seconds, seven and a quarter seconds being required for the wiping of each tentacle.

The feeding reaction usually occurred only after an individual had been undisturbed for some time and when it was partly buried in the mud or sand, but animals were sometimes observed to feed when attached to the side of a jar. No stimulus was found which would cause *Thyone* to extend the tentacles and feed. Attempts were made to induce animals to perform the feeding reaction by allowing crab or fish extract to flow gently over the anterior end; and by using mud from the place where they were collected, but such stimuli were without results or caused only the withdrawing reaction. In one instance, however, a positive reaction was observed. In this case a small portion of a bryozoan colony (*Bugula*) was dropped in such a way that it fell upon the anterior end of a partly buried individual. The tentacles were at once extended and the anterior end of the body was bent over so that they scraped the point where the stimulation had occurred. This experiment was repeated many times and on different individuals but no other positive reaction was induced. From these observations it may be concluded that the feeding reaction occurs only after the animal has been undisturbed for a time and is probably brought about mostly by internal factors, such as hunger.

Thyone's food consists of the microscopic organisms and debris to which the tips of the branching tentacles adhere. Such materials are wiped off as the tentacles are thrust into the mouth and extended again. The stomachs of seven freshly collected individuals were examined on August 10 and found to contain: living protozoans (*Lichnophora*, *Gymnodinium*), nematodes and diatoms (several species); filamentous and unicellular algæ; pieces of plant tissue; encysted protozoans; two harpacticid copepods; and an ostracod. *Thyone* is apparently a rather indiscriminate feeder but sand was infrequent in the stomach contents and, though particles of sand were seen sticking to the tentacles as they entered the mouth, most of them were brought out again as the tentacles emerged.

VI. Respiratory Movements.

Thyone carries on a regular system of breathing movements by which water is taken into the cloacal chamber and expelled again. The general plan of this chamber is shown in median longitudinal section in Fig. 4. There are three openings from

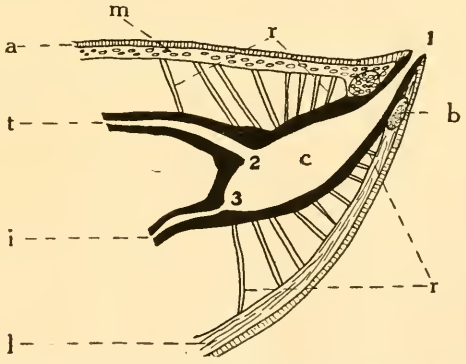

Fig. 4. Diagram representing a median longitudinal section through the posterior end of the body. *a*, integument; *b*, sphincter muscle; *c*, cloacal chamber; *i*, intestine; *l*, longitudinal body muscle; *m*, circular body muscles; *r*, radial muscles; *t*, respiratory trees. *1*, opening into cloacal chamber; *2*, opening into respiratory trees; *3*, opening from intestine.

the cloacal chamber *c*. These lead to the exterior *1*, to the respiratory trees *2*, and to the intestine *3*. During respiration the opening from the intestine *3* into the cloaca usually remains closed and takes no part in the breathing movements. Water is

drawn into the cloacal chamber by closing the opening to the respiratory trees *2* and contracting the radical muscles *r*, which extend from the cloaca to the body wall. The cloacal opening *1* is then closed, the respiratory tree aperture *2* is opened, and the contraction of the walls of the cloaca forces the water into the respiratory trees. Sometimes the cloacal opening *1* is kept closed while the water is forced back and forth from the cloaca to the respiratory trees, but the water is usually expelled from the body after each inspiration. When an individual is placed in shallow water so that the terminal opening *1* is just below the surface, the water is often expelled with enough force to form a fountain-like "spout" 3 or 4 cm. high.

The rate of the spouting reactions varies considerably, as is shown by the following observations, made upon two individuals which were buried in the sand. The average time between 144 consecutive spouting movements was 39 seconds for one animal; and the average time between 24 spouts was thirteen seconds for the other. In order to ascertain what would happen if these two individuals were prevented from spouting for a long period of time, they were first observed as they lay buried in the sand and the rate of their normal respiration noted. They were then made to pull the posterior end down into the sand by poking it with a glass rod, and whenever it started to emerge it was poked so that it was again withdrawn. The first animal spouted every twenty seconds (seven times) before being prevented from breathing and was then kept under the sand for one hour and twenty minutes. After the posterior end had appeared again there were no spouting movements for four minutes, and the next seven spouts averaged 36 seconds apart. The other animal averaged thirteen seconds between 24 spouts before its breathing was prevented. It was kept under the sand for two hours, 36 minutes and forty seconds; and at the end of that time it spouted with the posterior end still buried in the sand. The next nineteen spouts averaged one minute and five seconds apart. In both these instances the rate of respiration was more rapid before the breathing movements were prevented than afterwards. That is, an individual breathed more slowly after it had been made to "hold its breath" for an hour or two than it had before. This result can be accounted

for by the fact that the movements which force the water into the respiratory trees were more forcible after the period passed without respiration and hence they required a longer time. In addition to the respiratory movements described there is doubtless an exchange of gases through the integument and *Thyone* could probably exist for some little time without spouting.

VII. Responses to Stimulation.

Having completed the consideration of locomotion, feeding, and respiration, attention will now be directed toward some of the responses which result from such forms of stimulation as can be controlled by the experimenter.

1. *Tactile Stimulation.* — *Thyone* is extremely sensitive to contact with solid objects. If an animal is twisting about on the surface of the sand and comes in contact with a solid surface, the tube-feet are immediately extended and attached. Furthermore, if an individual is placed in a glass dish, it comes to rest in the angle between the bottom and side, where the body has the greatest surface in contact. When the contact stimulus is received from a moving object, the characteristic withdrawing reaction is given and the response varies with the stimulus. The tip of a glass rod may be gently pressed against the side of an individual if the movement is very gradual but the same pressure will cause a marked response if suddenly applied. The following experiment is a good example of sensitiveness to jars and other slight disturbances. A drop of water was allowed to fall from a height of one meter into a one-liter beaker containing a feeding individual. As soon as the drop struck the surface of the water above the animal, the tentacles were withdrawn and the cloacal opening was closed.

There is great variability in the sensitiveness of different individuals and those which had been in the laboratory for some time often allowed the tentacles to be touched with a glass rod whereas freshly collected individuals would contract at any slight jar, such as the closing of a door. Grave (:05) obtained similar results from his study of *Cucumaria*.

2. *Gravity.* — *Thyone's* responses to gravity were tested in two ways, by the righting reaction and by locomotion on an inclined

surface. The righting reaction is one of the most characteristic activities of this species. If an individual is placed in a flat-bottomed dish containing sea water and held with its ventral side uppermost until the tube-feet have attached themselves (usually about half a minute), it slowly pulls the body over with the tube-feet until the ventral surface is against the bottom of the dish. The tube-feet are helped to perform this righting reaction by the rings of muscular constriction which pass slowly from one end of the body to the other. The direction of the turning is determined by various factors, light being an important one. For example, when an individual rests on its dorsal surface with the long axis of the body at right angles to the direction of the light, the ventral surface is usually turned away from the light as the body is righted.

In order to test the locomotor reactions on an inclined surface four individuals were each given four consecutive trials in the

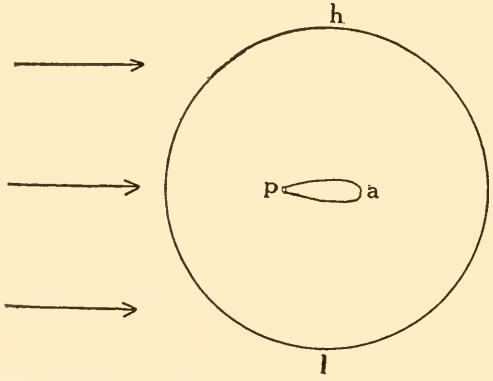

FIG. 5. Diagram showing the position in which individuals were placed in the dish during experiments to test the locomotion on an inclined surface. The arrows represent the direction of the light. *a*, anterior end ; *h*, high side of dish ; *l*, low side of dish ; *p*, posterior end.

bottom of a round glass dish, which measured thirty centimeters in diameter and contained sea water. This dish was placed directly in front of a window which was the only source of light in the room and the bottom was tipped 5.6° from the horizontal at right angles to the direction of the light rays. Animals were placed separately in this dish with the long axis of the body parallel to rays of light (Fig. 5). They were first given two trials with the bottom of the dish

inclined toward the left, the anterior end of the body being placed toward the window in the first case and away from it in the second. The dish was then inclined toward the right and the same procedure repeated. The distance from the center to the edge of the dish was fourteen centimeters and directions which the different individuals took in reaching the latter are shown in Table I. These results do not show any strongly geotropic tendency and the next step would naturally have been to test individuals on an inclined surface with the light coming from above or to make similar tests in total darkness but lack of time prevented such experiments being carried out.

TABLE I.

Deflection in moving fourteen centimeters away from the light on a plane surface inclined 5.6° at right angles to the direction of the light rays.

Direction of Locomotion.	Straight away from Light.	Up Incline.	Down Incline.
Individual No. 1		10°	
		10°	
	+		
			5°
Individual No. 2			90°
		90°	
		10°	
	+		
Individual No. 3	+		
	+		
	+		
	+		
Individual No. 4			10°
			22°
			10°
			5°
Total reactions	6	4	6
Average deflection	0	30°	24°

3. *Chemical Stimuli.* — No extensive experiments to test reactiveness to chemical substances were made. It was hoped, however, that positive responses might be obtained by using food substances, but repeated experiments with fish and crab extract, crushed eel-grass and scrapings from the surface of the mud were without results. The reactions which resulted when the water became foul were doubtless due to chemical stimuli. In this case individuals extended the posterior end of the body until the cloa-

cal opening was near the surface of the water, or came out of the sand and climbed up the side of the jar in which they were kept, or increased the size of the body and ceased to respond to shadows and other slight stimuli, and in some cases even cast out the visceral organs.

4. *Change in Density of Medium.* — *Thyone* can stand a marked increase or decrease in the density of the water in which it lives without serious interference with its activities. In order to test the effect of increased density an individual was placed in a one-liter beaker which had sand in the bottom to a depth of six centimeters. This beaker was filled with sea water and allowed to remain on the table in the laboratory from July 12 until August 7 (twenty-five days), when the animal died. During this time the water had evaporated so that the specific gravity had increased from 1.024 to 1.052. This animal showed no signs of the changes due to unfavorable environment until July 1, when it came out of the sand where it had been previously buried and lay on top. From this time it began to show signs of degeneracy, the skin was blistered off in spots and the body took on a peculiar elongated form. Many of the reactions continued to be normal however and the usual shadow reaction (which is described in the next section, p. 277) was easily induced the day before it died. On the morning of August 7 the animal failed to respond to shadows but gave the withdrawing response when it was poked gently. Four hours later it was dead. Subsequent examination showed that the visceral organs were still in place and had not been cast out on account of the increased density of the water.

The effects of a decrease in density were next investigated. Individuals were placed for a time in various mixtures of sea and fresh water. They were then returned to sea water and their subsequent condition noted. The results of these experiments are shown in Table II. No individual ever attempted to burrow while it was in a solution of lesser density than sea water, although sand was always placed in the bottom of the dish. The body remained contracted, spouting was infrequent and the tube-feet were never attached and seldom even extended over much of the body surface. In the cases where the animals survived their immersion in the mixtures they soon began regular spouting move-

ments after being replaced in sea water and gave typical, burrowing, feeding and shadow reactions within a few hours. The visceral organs were cast out in only one case and that was after an individual had been left in perfectly fresh water for three hours.

TABLE II.

Results of experiments in which *Thyone* was placed in mixtures of fresh and sea water.

Parts, by Volume, used for Mixture.		Number of Individuals Used.	Time Left in Mixture.	Condition after the Experiment.
Sea Water.	Fresh Water.			
1	1	1	3 hours.	Good.
1	2	2	1 hour.	Both good.
1	2	2	4 hours.	Both good.
1	2	2	24 hours.	Both good.
1	3	2	1 hour.	Both lived, but were in poor condition.
1	4	3	Until dead.	Died in 5-6 hours.
0	1	2	3 hours.	Recovered somewhat, but died within 3 days.

As will be seen from the table, animals which were left for twenty-four hours in a solution which consisted of one third sea water and two thirds fresh water, were apparently uninjured; while individuals which were immersed in fresh water for three hours died. Mr. E. D. Congdon's observations on this species are of interest in this connection. He told the writer that he had found *Thyone briareus* at the mouths of rivers in water which was half salt and half fresh, as judged by the specific gravity, but it was never found any farther up rivers than that.

5. *Light Stimulation.* — *Thyone* is extremely sensitive to a decrease in the light intensity and what may be called the "shadow" reaction is one of its most characteristic responses. If an individual is resting quietly in the sand with only the posterior end of the body exposed and the experimenter's hand is passed between it and the window, it at once withdraws the visible portion of the body. This response is of course variable and it may not occur at all or it may be so pronounced that the animal completely disappears beneath the sand. The same withdrawal is induced if a shadow is thrown on the anterior end or even on one tentacle and a particularly sensitive individual was caused to contract by extending a pencil over the top of the beaker in which it lay.

Although such characteristic responses are given when the light intensity is decreased no reaction occurs when there is a corresponding increase. An individual will contract at once when an object is interposed between it and the light, but it gives no response if the object is removed after a time. Furthermore, when light from a large oil lamp or from the sun was suddenly reflected from a mirror on a feeding individual there was no response. This sensitiveness to decrease in the light intensity and lack of response to an increase is similar to the reactions observed by Hargitt (:06) in *Hydroides dianthus* and other annelids. Uexkull ('97) has also described striking shadow responses in sea-urchins.

Thyone gives well-marked locomotor responses to light which may be illustrated by the following experiment: Eight individuals were successively placed in a shallow rectangular glass dish which measured 29 cm. long by 25 cm. wide, and contained sea water. The dish was enclosed in a black box which had an opening at one end. This opening was directed toward the window so that light was admitted from only one direction. The animals were always placed with the long axis of the body at right angles to the light rays and the direction of the subsequent movement was then observed. In a series of twenty-four reactions the locomotion in every case carried the animal away from the light to the end of the dish, but there was no definite orientation of the body in relation to the light. In ten of these negative responses the anterior end was ahead as the individual moved; in nine instances the posterior end preceded the anterior; and in five the locomotion was straight toward the right or left. Not one of the eight individuals moved in every case with the anterior or posterior end in front.

The influence of the negative light response is also apparent in the righting reactions. When individuals were placed in the same position as in the experiments described in the last paragraph, except that the ventral side of the body was uppermost, the righting reaction usually carried the ventral surface away from the light. Two individuals were given fifteen trials each in the manner just described, the anterior end being turned alternately toward the right and left in successive trials. One of them turned four times toward the light in righting itself and

the other turned only three times in that direction. In other words, twenty three out of thirty reactions (77 per cent.) were away from the light.

Another reaction which shows a negatively phototropic response is apparent when an animal burrows next the side of a glass vessel. It never remains against the glass but moves out into the sand after it has covered itself. This action is without doubt due to light stimulation for an animal will remain indefinitely in contact with an opaque object, such as a stone.

These reactions show that the *Thyone* is sensitive to decreased light intensity, and that it is negatively phototropic but without any definite orientation of the body to the source of the illumination, or the direction of the rays. This lack of orientation is rather striking in a bilaterally symmetrical animal and it shows that the response is not brought about in this case by unequal stimulation on the right and left sides of the body.

6. *Heat Stimulation.* — *Thyone* was not found to be very responsive to temperature changes, and individuals lived for several days at room temperature (24–28° C.) without apparent injury. Attempts were made to induce reactions by local changes in the temperature. The method was to siphon boiling water or a mixture of ice and salt water through a small U-shaped glass tube which could be brought close to the surface of the individual to be tested. Although six different animals were each tried twice with the hot tube and twice with the cold tube by holding the tube less than a millimeter below the extended posterior end, not a single response was observed.

Attention was next turned to the effects of an increase or decrease in temperature which affected the whole body. To test the effect of increased temperature six individuals (which were buried in the sand at the bottom of separate beakers containing sea water) were placed two at a time on a sand-bath and slowly heated. All the animals became active after the temperature had reached 30° C. The tube-feet were waved about on all sides and the body began to execute irregular twisting movements which continued until the temperature was lowered again. Two of the individuals were slowly heated to 36.5° C., the time required to reach that temperature being one hour and thirteen

minutes. One of these animals soon died but the other was in excellent condition four days afterward. Two other animals were heated to 41° C. during two hours and thirty-eight minutes and both of them died, although one continued to contract slowly when poked for two days. The two remaining individuals were heated to a temperature of 37° C. during two hours and forty minutes. Next day they were both in excellent condition and gave good burrowing and shadow reactions.

In order to test the effect of decreased temperature, beakers containing buried individuals were placed in a pail of cracked ice and salt and allowed to remain until the temperature had been sufficiently lowered. A beaker containing one animal was placed in the "freezer" and when the temperature had reached + 8° C. it failed to give the shadow response but contracted somewhat when the beaker containing it was jarred. In two hours and twenty minutes the sand was frozen solid and covered over with ice crystals. A thermometer held against the body of the animal registered — .5° C. At this temperature the posterior end still contracted when poked. Twenty minutes later with the temperature at — 1.6° C. only a feeble contraction was induced by poking, and after thirty minutes more the whole body was stiff and apparently frozen solid. The animal was left an hour longer and became completely covered over with ice crystals. The beaker was then removed, after having been in the freezer four hours and twenty minutes. The animal was found to be dead after the ice thawed. Another beaker which contained two *Thyones* was introduced into the freezer. Both these individuals were buried in the sand and covered by sea water. The temperature was reduced so that the sand was frozen and a thermometer resting against one of the animals registered — 2° C. to — 3° C. for two hours and forty minutes. After having been in the freezer three hours and forty minutes the beaker was removed. Twelve hours later both the individuals it contained had cast out the viscera but they did not die and continued active for several days, though they were in poor condition and gave no shadow reaction. Grave (:05) observed that *Cucumaria* retracted the whole body during cold weather and Mr. George Gray had informed me that *Thyone* buries itself six or eight inches in the sand

during the winter. I had therefore expected to see the burrowing reaction take place as the temperature was reduced but all three of the individuals remained perfectly quiet as the ice formed around them and the posterior end was not withdrawn.

From the experiments described it is evident that *Thyone* is comparatively insensitive to thermic changes and that it is able to react through a wide range of temperatures. The maximum and minimum vital limits are in the neighborhood of 40° C. and 0° C. respectively.

VIII. Experiments to Determine whether the Integrity of the Nervous System is Essential to Reactions.

The classical work of Romanes ('85) showed that a single fifth of the body wall of a sea-urchin was able to carry on locomotion without any of the visceral organs. Such fragments executed righting reactions and showed the same positive phototropism which was characteristic of entire animals. Mead (:01) kept detached starfish arms alive for as much as three months and they retained their powers of locomotion and gave the usual righting reaction. Von Uexkull ('97) found that pieces of sea-urchin would react to mechanical stimulation but the responses to shadows depended on keeping the system of radial nerves intact. These and other observations show that the nervous system of the asteroids and echinoids is little centralized in some respects, though Jennings (:07) has recently described some remarkable instances of association in the starfish. Clark ('99) found that cutting the oral nerve ring made no appreciable difference in the reactions of *Synapta*. Henri (:03, :03*a*, :03*b*) showed that nerve centers exist in the radial nerve trunks of *Stichopus regalis* and that reflex muscle contractions could be induced through them by stimulating the skin. He states that such radial nerve centers control only a limited portion of the body musculature and that reflexes which involve more than one of the longitudinal muscles must pass through the oral nerve ring.

As has been stated, the nervous system of holothurians consists of a circum-oral ring which gives rise to five radial nerve trunks and these are connected through their finer branches which anastomose to some extent. This system has been modified some-

what to conform to the bilateral plan of structure and, as the radial nerves are not equally well developed, there is a dorso-ventral as well as an antero-posterior differentiation. In order to test the reactions of fragments of the body a series of experiments was carried out in which twelve *Thyones* were each divided into two approximately equal pieces by a transverse cut. The two pieces of each animal were laid in a dish of sea water before a window in the position shown in Fig. 6. The ventral side of

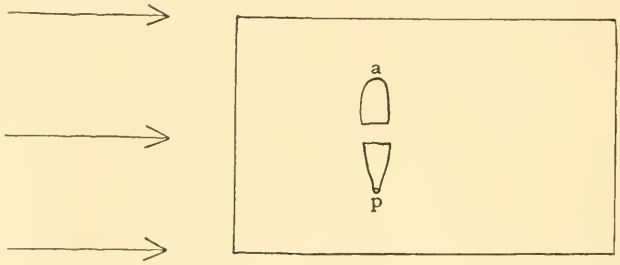

FIG. 6. Diagram to show the position in which animals were placed in the dish after being cut in two. The arrows represent the direction of the light. *a*, anterior end ; *p*, posterior end.

the body was always placed uppermost and the long axis of the body was at right angles to the direction of the rays of light. The movements of the two halves were observed during the three hours following their separation and a summary of these reactions is given in Table III. Although the tube-feet were more or less

TABLE III.

Showing the number of anterior and posterior halves of twelve *Thyones* which gave different reactions.

Nature of Reaction.	Locomotion.				Righting.	Shadow.
	Toward Light.	Away from Light.	Straight Ahead.	None.		
Number of anterior halves reacting.....	3	2	2	5	11	3
Number of posterior halves reacting.....	4	7	0	1	12	9

active on both halves of the body in all cases, it will be seen that the posterior halves gave characteristic reactions more often than the anterior portions. They carried on normal spouting move-

ments and performed the righting reaction more rapidly than the anterior halves. The only response in which the anterior half approached the posterior in the number of individuals which responded was in the righting reaction. This was probably due, in part at least, to the structure of the tube-feet which have more efficient sucking discs on the ventral than on the dorsal surface, and those on the ventral side would hence be more likely to become attached if all of them were active. Two of the individuals were kept in a dish without changing the water for three days after they had been bisected. Both the posterior halves remained in good condition and gave characteristic shadow responses at the end of the third day, but both the anterior portions threw out the viscera during the second day and were dead on the third day.

These experiments show that the presence of the circum-oral nerve ring is not essential for the performance of correlated reactions and that the posterior half of the body is apparently able to carry on movements better than the anterior. This greater efficiency shown by the posterior end of the body is perhaps what might be expected from the fact that the whole anterior portion is often cast off and dies while the posterior end lives and regenerates the lost organs. These conclusions do not agree with those reached by Henri (:03*b*) from his work with *Stichopus regalis*. He believed that the oral nerve ring was necessary for general muscular reflexes.

IX. Variability of Reactions.

Few of the reactions which have been described in this paper could always be induced by a repetition of a stimulus which had previously brought them about. The response which was perhaps the most unfailing was the contraction of the body which resulted from gentle mechanical stimulation with some pointed object, but even this response varied with the strength of the stimulus, the condition of the individual and other factors. Not only did characteristic responses often fail to take place after a stimulus but they were sometimes modified so that they did not take place in the usual manner. Such differences in reactions may be due to internal causes which have to do with the structure or the past experience of the individual, or they may be caused by various

external factors. It is important to discover, if possible, what stimuli will cause these differences in behavior. When *Thyone* extends its tentacles and feeds, the movements are brought about by such factors as hunger, or the presence of food, or by a combination of two or more such stimuli. If we throw a shadow on it as it feeds, the tentacles contract and the withdrawing reaction takes place. Although all the stimuli which were effective in producing the feeding reaction but a moment ago are present and acting, we have introduced an additional stimulus which has modified the response. This is an example of inhibition, as the presence of one stimulus inhibits the response to certain other stimuli. The periodic repetition of a stimulus is another means by which responses may be changed. As Jennings (:05) says of this method "the physiological state tends to resolve itself into another and different state" after a stimulus has been received. An individual will be in a different condition, and will really be a different animal, after it has received the first stimulus and may therefore give a different response the second time the same stimulus is received. Some of the instances of variable behavior which were observed will now be briefly considered.

1. *Repetition of a Stimulus.*— Responses usually vary in degree when a stimulus is repeated at regular intervals. If an individual is touched gently with a glass rod and then touched again on the same spot at one minute intervals the withdrawing response which was at first marked becomes gradually weaker and finally ceases altogether. Similar results may be obtained by allowing a drop of water to fall at regular intervals into the dish which holds an animal, or by periodically throwing a shadow upon an extended individual. By increasing the interval of time between successive stimuli a larger number of responses may be obtained but the result will be the same in the end. Individuals which have been newly brought from the ocean contract at the slightest disturbance and give the withdrawing reaction whenever anyone walks across the floor or opens a door or when any other slight change occurs in their surroundings, but they soon cease to respond to such stimuli. For example, one individual which had been kept for two weeks on a table in the laboratory carried on normal feeding and breathing reactions while people were con-

stantly passing between it and the window. After this animal had been allowed to remain in a quiet situation in another room for a week however it had again become extremely sensitive to shadows, jars, currents of water and other gentle forms of stimulation.

Thyone sometimes modified its behavior after a stimulus had been repeated several times and a new form of response occurred. On one instance an individual which had been used in previous experiments was stimulated by gently sticking a glass rod among the tentacles as it was feeding. At first all the tentacles were withdrawn as soon as the rod touched one of them, but after the fourth trial they were no longer retracted, and when the rod was pressed gently against the mouth the anterior end was turned to one side but not withdrawn. This change in response was brought about in half an hour.

2. *Inhibition.* — As has been stated, the shadow reaction was one of *Thyone's* most constant and characteristic types of response but it would not take place if certain stimuli were present. To give some specific instances : This reaction was inhibited when the temperature of the water fell below 10° C., when the posterior end was greatly elongated toward the surface on account of stagnant water, and after the respiratory movements had been prevented from occurring for some time. In all these cases individuals gave characteristic shadow responses before and after the inhibiting stimulus was present. Another characteristic reaction was locomotion away from the light, but, when an animal was against the side of a glass vessel it often moved at right angles to the direction of the light rays, the thigmotactic stimulus being more potent than the light. Furthermore, if an individual was laid on its dorsal surface with the median plane inclined slightly toward the source of the illumination, it often moved one or two centimeters toward the light in righting itself. These instances are typical of others which might be given and they show that though *Thyone's* responses are largely of a stereotyped nature, they are interrelated in such a way that one may inhibit another.

X. General Considerations.

Thyone briareus is a holothurian which is rather strikingly adapted to a sedentary life. It is not able to change its place of abode easily and it is hence highly resistant to unfavorable conditions in its environment. Individuals which were allowed to lie on moist sand exposed to the air for eighteen hours were apparently uninjured. This tenacity to life is also shown by the ability this species manifests to withstand changes in the temperature and the density of the water in which it lives. The methods of feeding, locomotion, respiration and other activities are adapted to the peculiar conditions under which it exists. Passing most of its life buried in the mud, *Thyone* probably does not often fall a prey to large enemies but it is protected from them by the withdrawing reaction, by its locomotion away from the light and by its habit of pulling pieces of eel grass and other debris over the body.

Many of *Thyone's* movements show a lack of correlation. In ordinary locomotion on a solid surface, the tube-feet which are behind are often forcibly pulled loose from their attachments instead of being released by means of some impulse from the central nervous system. Such organs as the tube-feet are able to work more or less independently, but they may also be actuated by a unified impulse, as is shown when they are simultaneously extended or contracted over the whole body and the same unity is apparent in their action as they pull the animal along in a definite direction. On the other hand many reactions show considerable power of correlation and adaptation. Correlation is shown in the use of the circum-oral tentacles, as they move in a rather definite order. Very often, however, two of them endeavor to enter the mouth at the same time, but one always bends aside to make way for the other. If the correlation in the movements was perfect in this case, two tentacles would not try to enter the mouth at once, and if there was no correlation they would struggle with each other indefinitely. Furthermore, when a feeding individual lies on its side, the tentacles which scrape the bottom are used oftener than the others and there is thus an increased chance of obtaining food.

Generally speaking, it may be said that *Thyone's* behavior,

like that of other sedentary animals, is mostly made up of stereotyped reactions which occur regularly in response to certain stimuli. Furthermore, many of these reactions are carried on independently by certain separate organs and two parts of the body may "work against each other" for a time, but, under the proper conditions of stimulation, all these simpler reactions may be unified into one general correlated response. Although the reactions are largely stereotyped in nature they may be changed by experience or inhibited by the presence of different stimuli. The stereotyped methods of response are usually adequate to meet the conditions under which *Thyone* exists and would usually enable it to survive in the struggle for existence. If they are not adequate, however, they may be modified to meet new conditions. For example, this species usually burrows into the mud so that only the posterior tip of the body is exposed and even this is withdrawn if the slightest shadow falls upon it or if the water is agitated. If the water becomes stagnant the same individuals that were formerly so reactive will climb up the side of the jar and cease to respond to such slight stimuli as shadows and water currents, and they contract only when touched by some solid object. As the water becomes foul, the greatest need of the organism is oxygen and the behavior described would enable this to be obtained, but to accomplish this end, the animal would be obliged to forego the temporarily less important matter of protection from its enemies.

When compared with an echinoid or a star-fish as described by Romanes ('85) and Jennings (:07), or with an ophurian as it is represented by Glaser (:07) *Thyone* falls short in the range and diversity of its reactions. This is probably due in part to its sedentary mode of existence and the study of holothurians which do not burrow might show a somewhat different set of reactions. Perhaps the most interesting point which is brought out in the study of *Thyone's* behavior is the fact that, although the symmetry is so strikingly bilateral, the locomotion is carried on with the same lack of orientation which is so characteristic of other groups of echinoderms.

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University of Michigan,
Ann Arbor, Michigan,
September, 1908.

THE INTERLOCKING MECHANISMS WHICH ARE FOUND IN CONNECTION WITH THE ELYTRA OF COLEOPTERA.

ROBERT S. BREED AND ELSIE F. BALL.

It is a matter of common knowledge that the elytra of most beetles are co-adapted with each other and with the body of the insect. Yet it appears that no one has ever described these co-adaptations in any detail in spite of the fact that several very interesting mechanical devices are here brought into use. The attention of the senior author of this paper was called to them while studying the muscular system of beetles. At that time he discovered that a small muscle, attached to the meta-episternum, had been erroneously thought to be an expiratory muscle and that in reality it operated a mechanism for hooking the edge of the elytron to the body at this place (Breed, :03, page 332).

Writings on the subject seem to be limited to a few general statements in text-books. Packard ('98) says that the wing cases of beetles join along their dorsal suture like the valves of a mussel shell. He further states that there is an interlocking of the elytra with the scutellum, citing the stag-beetle as an example. He also finds that the elytra of stag-beetles interlock with each other by means of a groove, and that this is the method usually found in beetles; but that in some cases the joining is after the method of two cog-wheels. He likens these devices to the two methods most used by the cabinet-maker in joining boards.

Sharp ('99) says that in most beetles the elytra are fitted together and to the sides of the body, except at the tip, but he gives no further explanation. He also states that sometimes the tips of the elytra are fastened to the body, but that this occurs only in the cases where the abdomen is not entirely covered by them. He says further that in the blister beetles, which include the Cantharides and the Meloides, the elytra are not co-adapted with the abdomen. The former are winged but the latter are so-called

apterous forms, with elytra overlapping at the base. The same author says that in some species the elytra are soldered together along the suture. The degree of firmness of the joining varies even in specimens of the same species, probably depending on the age of the individual.

1. Material and Methods.

The material used was *Lachnosterna fusca* Auct., the June-bug or May-beetle, *Thymalus marginicollis* Chevr., a small beetle which lives in the common shelf fungus of white birch, and *Tenebrio molitor* Linn., the meal beetle.

The method used in examining the co-adaptations of the elytra in the two larger beetles was to take specimens hardened in alcohol and cut off the posterior part of the body, wings and elytra with a razor. Then the remainder of the insect was placed under a low power of the microscope in such a position that the face of the cross section could be viewed. In some cases it was found advantageous to embed the whole beetle in paraffin first. Then the desired section was cut free-hand with a razor, after which a part or the whole of the paraffin was dissolved away and the cut face examined with a microscope. When studying the separate elytra, thin sections were cut in pith in the same way that botanical sections are so often prepared. In this way perfect, though rather thick, sections were obtained, whereas microtome sections were badly broken.

In the case of *Thymalus*, which is a small beetle with a comparatively thin cuticula, it was found possible to prepare series of microtome sections of the entire insect. These series show both the interlocking along the dorsal suture and the musculus episternalis with its related parts. By selecting young imagines of *Lachnosterna* and *Tenebrio* and sectioning only a portion of the body, series of microtome sections of these beetles were prepared which show the musculus episternalis and its related parts.

2. Observations.

There are commonly found in beetles four devices for fastening the elytra in place, all of which may be utilized in one animal. The fastening may be accomplished :

1. By a co-adaptation of the elytra along the dorsal suture.

2. By means of a groove on the dorsal face of the metathorax into which the swollen inner edges of the elytra fit.

3. By slipping the anterior edges of the elytra under the scutellum and hooking them (*a*) on to the scutellum, or (*b*) on to the metathorax. Pressure derived from the retracted prothorax may aid in keeping these edges in position.

4. By hooking the anterior lateral edges of the elytra over ridges or into grooves on the lateral faces of the metathorax.

(*a*) *Interlocking Mechanisms found in Lachnosterna.*

Lachnosterna fusca is one of the lamellicorn beetles. The head, prothorax and elytra show in dorsal view, together forming a broad oval outline. The elytra almost cover the abdomen, the exposed part being curved downward in such a way that it cannot be seen from above. The abdomen is shortened and rounded, and the elytra curve sharply downward at the sides and posteriorly.

The first three of the methods of interlocking mentioned above are used in *Lachnosterna*. The hooking mechanism of the fourth method is present, but is not functional.

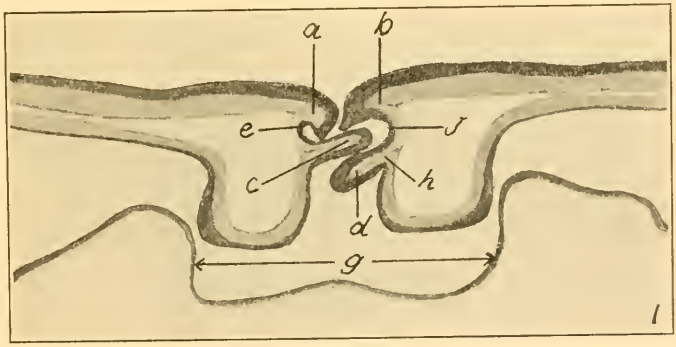

FIG. 1. Mid-dorsal portion of the cross section of the elytra and metathorax of *Lachnosterna*, showing the adaptation of the elytra to each other along the dorsal suture and to the metathoracic groove. ×75.

1. The method of joining along the dorsal suture is shown in Fig. 1, which represents a cross section of the mid-dorsal region of the elytra. In this case the elytra are nearly as they would be when the elytra were firmly closed, and are partially slipped into

the underlying metathoracic groove. When the elytra are separated, the cross section of the right one appears as in Fig. 2. The ridge *b* is stiff, but the ridge *d*, being narrower at *h*, acts like a spring. When the two elytra are drawn together, the ridge *c* strikes the ridge *d* and bends it downward. The ridge *c* then

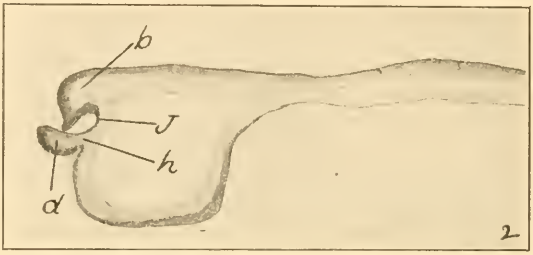

FIG. 2. Cross section of the mid-dorsal portion of the right elytron of *Lachnosterna*, showing the relation of the two ridges when the two elytra are not locked together. ×75.

slips into the groove *j*, and *d* springs back, holding *c* tightly in the groove *j*. By comparing Fig. 2 (a figure of the right elytron) with that of the left elytron represented in Fig. 1, it will be seen that the two are remarkably alike when the elytra are separated. No conception of the way in which they interlock was formed until the two were studied in their natural relations by the method

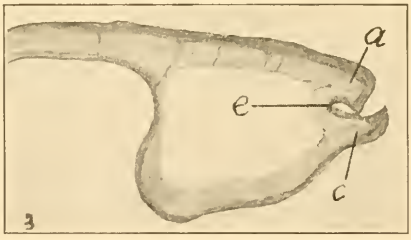

FIG. 3. Cross section of the mid-dorsal edge of the left elytron of *Lachnosterna* as seen in the extreme anterior portion. ×75.

described above. The ridges and grooves along the dorsal suture have essentially the same form throughout the length of the elytra but the joining is the firmest along the anterior third of the suture. Figs. 3 and 4 represent the region close to the point of the scutellum. Figs. 1 and 2 show the structure at a point

about one millimeter back of the scutellum. Figs. 5 and 6 show cross sections of the elytra from near the middle region. In all of the specimens examined, the ridge on the left elytron hooks into the groove on the right elytron.

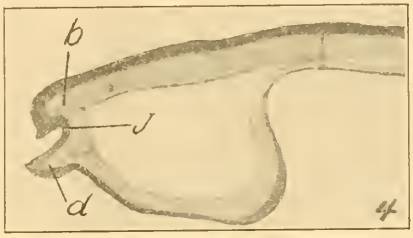

FIG. 4. Similar to Fig. 3. Right elytron. ×75.

2. Figs. 1 to 6 show the thickenings of the lower face of each elytron along the mid-dorsal edge in cross section. Figs. 1 and 2 show the form of these thickenings in the metathoracic region. Beneath these thickenings there is a groove along the metathorax, well stiffened with chitin, into which the thickenings fit tightly when they are interlocked. This groove is seen in cross section at *g*, in Fig. 1. The thickenings of the elytra in the

FIG. 5. Cross section of the mid-dorsal thickening of the left elytron of *Lachnosterna* as seen near the posterior end of the elytron. ×75.

anterior part of the suture are more abrupt than elsewhere, the contour of the under side of the elytron being here S-shaped in cross section (Figs. 3 and 4), so that the thickenings hook into the metathoracic groove, which is slightly wider in its ventral than in its dorsal part.

3. The metathoracic groove widens in its anterior portion, so that the entire groove has the form of a Y with its arms projecting forward. The triangular scutellum lies in the opening between the arms of the Y. In closing the elytra, their anterior

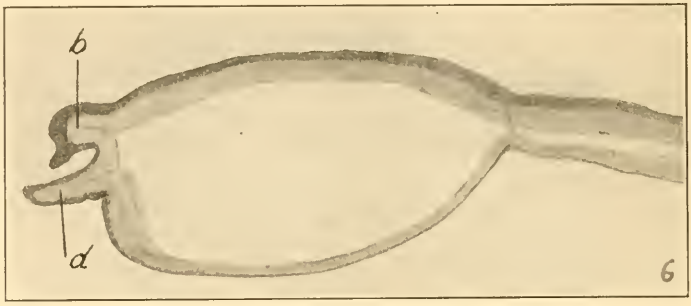

FIG. 6. Similar to Fig. 5. Right elytron. ×75.

edges slip under the diagonal edges of the scutellum and hook over the ridges made by the diverging arms of the Y. The thickening along the edge of the elytron at this point has still

FIG. 7. Cross section of the lateral edge of the right elytron of *Lachnosterna*. ×150.

more the form of a hook than those shown in Figs. 3 and 4. The interlocking is therefore very firm in this region, especially as the downward pressure of the scutellum aids in resisting any

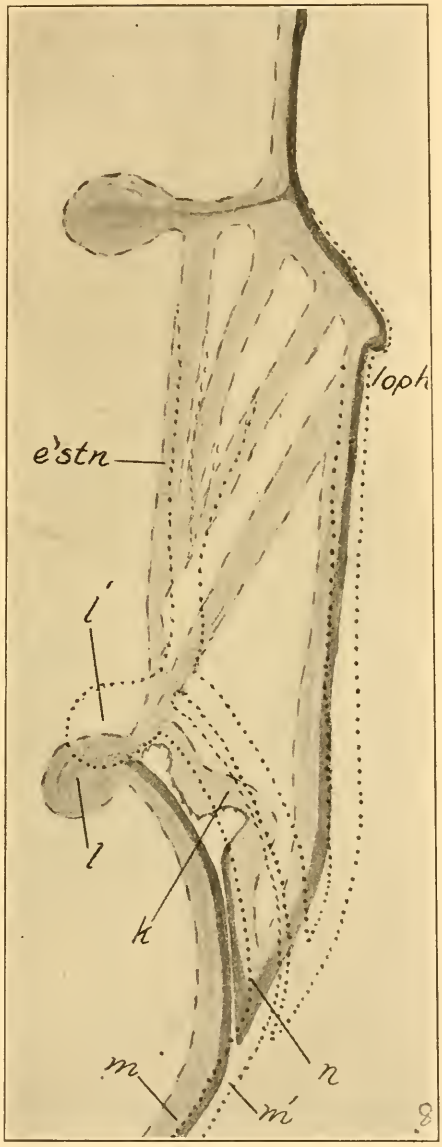

FIG. 8. Lateral portion of a cross section of the metathorax of *Lachnosterna*, showing the parts affected by the contraction of musculus episternalis. × 150.

forcible detachment of the elytra. This pressure of the scutellum is supplemented by the retraction of the prothorax against the

mesothorax. There is no interlocking of the elytra with the scutellum in this beetle.

4. The elytra are not fastened along the lateral faces of the metathorax, although a rudimentary hook (Fig. 7) is found along the anterior lateral edge of each elytron. A corresponding ridge hooking downward (Fig. 8, *loph.*) is found on the meta-episternum, a short distance below the origin of the musculus episternalis (*e'stn.*).

The probable method by which this hook would be operated is shown in Fig. 8. The dotted lines show the position of the movable parts when the muscle is contracted. The ridge represented in cross section at *l* is drawn up to the position *l'*, and the flexible band *k* is straightened. The meta-sternum *m* is drawn upward and outward to the position *m'*, thus forcing the ventral edge *n* of the episternum outward. A dissection of the muscular system of several beetles has shown that the lower attachment of the musculus episternalis is along a straight line, while the upper attachment is arched somewhat like the gable of a house. This form gives firmness and affords reason for believing that it is the lower attachment of this muscle which moves when the muscle contracts. Further proof of this is furnished by the triangular form of this muscle in longitudinal section (Fig. 8, *e'stn.*). Thus the dorsal end of this muscle serves as origin, while the ventral end is insertion. The movement of the ventral edge of the episternum outward would cause the slant of the ridge (*loph.*) to change slightly. If there were a functional hook present on the edge of the elytron, the change in position due to the contraction of this muscle would be sufficient to release the elytron or to allow it to return to its place.

However, since the hook present on the elytron is rudimentary, this muscle is apparently functionless though it must have been functional in some ancestral form. The muscle has not degenerated as completely as have the chitinous structures in connection with it. The degeneration of the lateral hooking mechanism may be accounted for by the highly developed interlocking mechanisms in the other parts of the body. The dorsal suture, the meta-thoracic groove, and the fastening under the scutellum furnish ample means for holding the elytra firmly in place.

(*b*) *Interlocking Mechanisms found in Thymalus.*

Thymalus marginicollis is one of the trogositid beetles ; it has the same general form as a lady beetle, but it is still better adapted in form of body for clinging to a surface after the manner of a limpet. The dorsal view of the body is a nearly perfect broad oval showing simply the pronotum and elytra with a small portion of the head. The flange on the edge of the elytron (seen at *f* in Fig. 9) fits closely against the surface on which the beetle is resting. The adult beetles are commonly found during the

FIG. 9. *Thymalus.* Copied from Breed, :03. Similar to Figs. 8 and 12. × 100.

early part of the summer lurking about the shelf fungi which have served their larvæ as food.

The four general methods of fastening the elytra in place which were mentioned above are all functional in this beetle. The co-adaptation between the body and the elytra is so perfect that it is nearly impossible to unclasp the elytra in a dead specimen without tearing them.

1. The co-adaptation along the dorsal suture is shown in Figs. 10 and 11. The lower ridge *d* (Fig. 11) of the right elytron fits into the groove *e*, the interlocking in this case being just the reverse of that found in *Lachnosterna* (Fig. 1). Moreover, there is no clasp arrangement, the ridge *c* remaining as rigid as the others. The suture would not hold together if there were not a lateral stress exerted upon the elytra. In the anterior region,

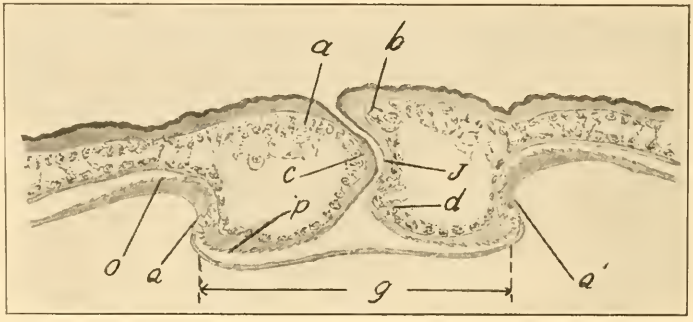

FIG. 10. Mid-dorsal region of a cross section of the elytra and metathorax of *Thymalus*, showing the adaptation of the elytra to each other and to the metathoracic groove. × 130.

the ridges become very feeble and in the most anterior sections (see Fig. 10) a ridge *c* on the left elytron fits into a groove *i* on the right elytron thus reversing the condition found in the middle and the posterior regions.

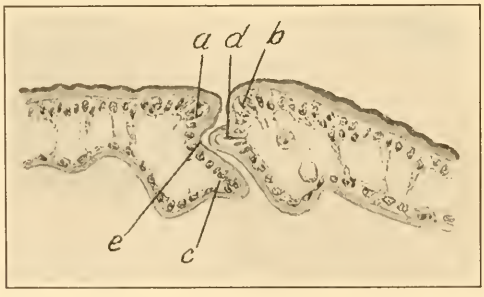

FIG. 11. Mid-dorsal region of a cross section of the elytra of *Thymalus* in the region immediately posterior to the metathorax. × 130.

2. Fig. 10 shows in cross section the thickened edges of the elytra, and the metathoracic groove (*g*) into which they hook.

The hooks are so pronounced that they make a very perfect joining, which holds the edges of the elytra together along the dorsal suture. This arrangement is made more effective by rows of minute chitinous teeth (*o*, *p*) which in the cross section resemble saw teeth. These are so directed that they keep the elytra from slipping laterally when they are being drawn together, by interlocking at the points *q* and *q'* they assist in keeping the wing covers perfectly co-adapted when at rest.

3. The interlocking of the elytra of *Thymalus* along their anterior edges is very much as it is in *Lachnosterna*. The principal difference is that the inner anterior corners of the elytra slip under the scutellum more than in the June beetle and the pronotum fits against the anterior edges more firmly.

4. The method of fastening the elytra to the meta-episternum is shown in Fig. 9. This figure is copied from Breed (:03, Fig. 13, Plate 6). A description of the mechanism which it illustrates is to be found on page 332 of the paper referred to. There is a ridge (*pli.*) on the face of the episternum, lying between the attachments of the musculus episternalis (*e'stn.*). The contraction of this muscle causes the ridge (*pli.*) to take the position indicated by the dotted line, thus freeing the ridge (*loph.*) on the elytron and allowing the latter to be raised. In like manner the musculus episternalis contracts when the elytron returns to rest and its immediate relaxation causes the latter to be securely fastened in its place. At the point marked by a star (*) there is found on the inner face of the elytron a row of minute teeth directed upward. A similar series of teeth pointing downward is found on the body wall opposite. These teeth interlock and assist in keeping the elytron in place. A few small teeth are also present on the inner face of the ridge (*pli.*).

The dorsal suture is thus kept intact by the combined working of the dorsal metathoracic groove and this lateral hooking arrangement. The combination of these two devices produces the stress which holds the mid-dorsal edges of the elytra in the positions shown in Figs. 10 and 11.

(*c*) *Interlocking Mechanisms found in Tenebrio.*

The common meal beetle is one of the Tenebrionidæ. It may be found abundantly through the summer months in granaries and mills, or flying into houses. It is an elongated beetle whose head, prothoracic and body regions are distinctly separated from each other in dorsal view.

The elytra of this beetle are not very firmly interlocked either with the body or with each other. All four of the methods previously mentioned are used in this interlocking.

1. The mid-dorsal edges of the elytra are co-adapted much as in *Lachnosterna* (cf. Fig. 1). However minute teeth are found along the dorsal surface of the ridge *c*, which fit in with similar teeth on the ventral side of the ridge *b*. In three of the individuals examined the ridge on the right elytron fitted into a groove on the left elytron while in two others the reverse was true.

2. The dorsal groove along the metathorax is both shallow and narrow. Minute interlocking teeth are developed on the thickened edges of the elytra and on the metathorax, as in *Thymalus* (cf. Fig. 10). These teeth are more blunt than those in *Thymalus* and do not form as perfect an interlocking device.

3. The inner anterior corners of the elytra slip under the diagonal edges of the scutellum, showing very perfect co-adaptation at this point. The prothorax does not fit tightly against the elytra and is not used in holding them in place.

4. The most interesting of these co-adaptations is that of the lateral edges of the elytra with the episternum. As seen in Fig. 12, which represents the right lateral portion of a cross section through this region viewed from behind, there are numerous teeth on the inner surface of the elytron at *s*, which interlock with teeth on the body wall at *s'*. Apparently the teeth at *t* and *t'* do not interlock because: (1) long movable hairs are found along the small ridge *u*, which would interfere with this action; (2) the shape of the body would prevent it; (3) the teeth themselves do not seem stout enough to serve for this interlocking. The action of the teeth at *s* and *s'*, working in connection with the co-adaptations along the dorsal suture, would cause a strain on the elytra which would hold them in place.

The triangular area embraced between the straight dotted lines shows the form of the episternal muscle projected on the plane of the section. The origin of the musculus episternalis is along the entire dorsal boundary of the meta-episternum. Most of its fibers take their origin from the thickened ridge *v*. The insertion is by means of a tendon which attaches to the anterior portion of the ventral edge of the episternum. The entire muscle is thus

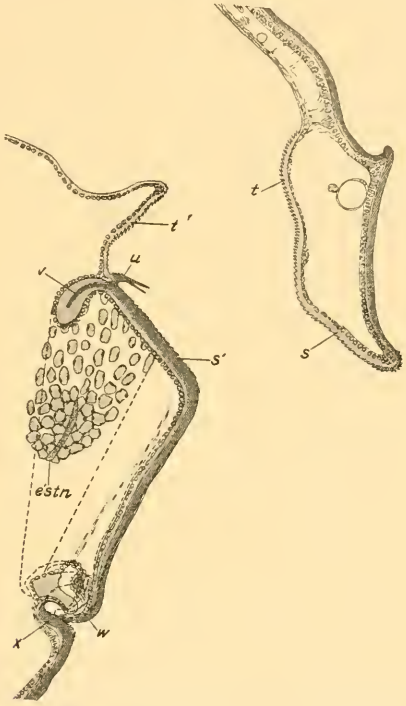

FIG. 12. Lateral portion of a cross section of the metathorax of *Tenebrio*, showing the parts affected by the contraction of musculus episternalis. × 75.

somewhat fan-shaped in side view. It is only in sections anterior to the one shown that the fibers are cut so as to show their full length in any one section.

It is difficult to determine the exact method by which the muscle operates. The structure of the suture to which this muscle attaches is remarkably complex and entirely unlike that found in the two beetles just described. The structure shown in Fig. 12 is typical for all of the sections of this suture The teeth shown

along the outer surface of the region *w* and the surface *x* show a better development in the section figured than in most of the sections.

There are at least two reasons for thinking that the condition figured is one which represents the contracted or nearly contracted state of this muscle. These reasons are: (1) the cross striations of the muscle appear as they do in contracted fibers, (2) the position of the parts affected. A dotted outline has been drawn (not shown satisfactorily in the figure) which indicates the probable position of these parts when the muscle relaxes. This relaxation would cause the teeth at *s* and *s'* to grip each other firmly if the elytron had previously been brought near enough the body for these surfaces to touch each other.

The most effective of the interlocking devices in this beetle are the mid-dorsal metathoracic groove, the slipping of the corners of the elytra under the scutellum, and the interlocking of these teeth along the meta-episternum.

(*d*) *Comparison.*

A comparison of the devices which are used by these beetles in holding their elytra in place reveals a close similarity in all cases, except in the interlocking of the lateral edges with the meta-episternum. Here the most striking dissimilarity exists. In all of the beetles which we have examined, a muscle has been found which originates along the dorsal edge of the episternum, and is inserted on the suture which marks the ventral boundary of this plate. This has been called the musculus episternalis. In one case (*Lachnosterna*) this muscle is apparently functionless since the chitinous structures which it operates are so degenerate that they no longer interlock. In *Thymalus* this muscle operates an upward hooking ridge, in *Tenebrio* a series of downward hooking teeth.

This dissimilarity of structure with its consequent differences in the method of operation is made possible by varying flexibilities of the chitinous cuticula.

It would be interesting to know how many other variations of these structures may be present among beetles. The three species examined were chosen at random and it does not seem possible

that all of the variations have been discovered. Some light might be thrown on the relationships of the various families of beetles to one another if more were known about these interlocking devices.

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